INTRACELLULAR SIGNALING MOLECULES

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of intracellular signaling molecules and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative, autoimmune/inflammatory, neurological, gastrointestinal, reproductive, and developmental disorders.

BACKGROUND OF THE INVENTION

Cell-cell communication is essential for the growth, development, and survival of multicellular organisms. Cells communicate by sending and receiving molecular signals. An example of a molecular signal is a growth factor, which binds and activates a specific transmembrane receptor on the surface of a target cell. The activated receptor transduces the signal intracellularly, thus initiating a cascade of biochemical reactions that ultimately affect gene transcription and cell cycle progression in the target cell.

Intracellular signaling is the process by which cells respond to extracellular signals (hormones, neurotransmitters, growth and differentiation factors, etc.) through a cascade of biochemical reactions that begins with the binding of a signaling molecule to a cell membrane receptor and ends with the activation of an intracellular target molecule. Intermediate steps in the process involve the activation of various cytoplasmic proteins by phosphorylation via protein kinases, and their deactivation by protein phosphatases, and the eventual translocation of some of these activated proteins to the cell nucleus where the transcription of specific genes is triggered. The intracellular signaling process regulates all types of cell functions including cell proliferation, cell differentiation, and gene transcription, and involves a diversity of molecules including protein kinases and phosphatases, and second messenger molecules such as cyclic nucleotides, calcium-calmodulin, inositol, and various mitogens that regulate protein phosphorylation.

Intracellular signaling is carried out by a variety of molecules that promote the transduction and amplification of the signal. For example, binding of a ligand to a transmembrane receptor activates membrane-associated intracellular proteins, such as G-proteins. G-proteins mediate both the level of intracellular second messengers, such as cyclic AMP, and the activity of signaling enzymes, such as phospholipase C. These messengers and enzymes then activate signal transduction pathways, many of which are mediated by protein kinase cascades. Phosphorylation of proteins in response to extracellular signals, cell cycle checkpoints, and environmental or nutritional stresses is often accomplished by transfer of a high energy phosphate from ATP. Second messengers whose effects are mediated by protein kinases include cyclic AMP, cyclic GMP, inositol triphosphate, cyclic ADP

ribose, and calcium/calmodulin. Alternatively, binding of ligand to a transmembrane receptor, such as a receptor tyrosine kinase, triggers the activation of a molecular "switch," such as a monomeric GTPase. In this case, binding of ligand to the receptor activates a catalytic domain in the intracellular portion of the receptor. This activated domain then switches on the activity of monomeric GTPases such as Ras, usually via adaptor proteins.

Cells also respond to changing conditions by switching off signals. Many signal transduction proteins are short-lived and rapidly targeted for degradation by covalent ligation to ubiquitin, a highly conserved small protein. Cells also maintain mechanisms to monitor changes in the concentration of denatured or unfolded proteins in membrane-bound extracytoplasmic compartments, including a transmembrane receptor that monitors the concentration of available chaperone molecules in the endoplasmic reticulum and transmits a signal to the cytosol to activate the transcription of nuclear genes encoding chaperones in the endoplasmic reticulum.

Certain proteins in intracellular signaling pathways serve to link or cluster other proteins involved in the signaling cascade. These proteins are referred to as scaffold, anchoring, or adaptor proteins. (For review, see Pawson, T., and Scott, J.D. (1997) Science 278:2075-2080.) As many intracellular signaling proteins such as protein kinases and phosphatases have relatively broad substrate specificities, the adaptors help to organize the component signaling proteins into specific biocehmical pathways.

Gangliosides, generally associated with plasma membranes, also participate in signal transduction. Aberrant ganglioside function has been implicated in inflammatory and degenerative diseases within and outside of the nervous system, including Tay-Sachs disease, multiple sclerosis, lupus erythematosus, and insulin-dependent diabetes mellitus (Misasi, R. et al. (1997) Diabetes Metab. Rev. 13:163-179).

Many of the above signaling molecules are characterized by the presence of particular domains that promote protein-protein interactions. A sampling of these domains is discussed below, along with other important intracellular messengers.

Intracellular Signaling Second Messenger Molecules

Phospholipid and Inositol-phosphate Signaling

Inositol phospholipids (phosphoinositides) are involved in an intracellular signaling pathway that begins with binding of a signaling molecule to a G-protein linked receptor in the plasma membrane. This leads to the phosphorylation of phosphatidylinositol (PI) residues on the inner side of the plasma membrane to the biphosphate state (PIP_2) by inositol kinases. Simultaneously, the G-protein linked receptor binding stimulates a trimeric G-protein which in turn activates a phosphoinositide-specific phospholipase C-β. Phospholipase C-β then cleaves PIP_2 into two

products, inositol triphosphate (IP_3) and diacylglycerol. These two products act as mediators for separate signaling events. IP_3 diffuses through the plasma membrane to induce calcium release from the endoplasmic reticulum (ER), while diaacylglycerol remains in the membrane and helps activate protein kinase C, an STK that phosphorylates selected proteins in the target cell. The calcium response initiated by IP_3 is terminated by the dephosphorylation of IP_3 by specific inositol phosphatases. Cellular responses that are mediated by this pathway are glycogen breakdown in the liver in response to vasopressin, smooth muscle contraction in response to acetylcholine, and thrombin-induced platelet aggregation.

Cyclic Nucleotide Signaling

Cyclic nucleotides (cAMP and cGMP) function as intracellular second messengers to transduce a variety of extracellular signals including hormones, light, and neurotransmitters. In particular , cyclic-AMP dependent protein kinases (PKA) are thought to account for all of the effects of cAMP in most mammalian cells, including various hormone-induced cellular responses. Visual excitation and the phototransmission of light signals in the eye is controlled by cyclic-GMP regulated, Ca^{2+}-specific channels. Because of the importance of cellular levels of cyclic nucleotides in mediating these various responses, regulating the synthesis and breakdown of cyclic nucleotides is an important matter. Thus adenylyl cyclase, which synthesizes cAMP from AMP, is activated to increase cAMP levels in muscle by binding of adrenaline to β-andrenergic receptors, while activation of guanylate cyclase and increased cGMP levels in photoreceptors leads to reopening of the Ca^{2+}-specific channels and recovery of the dark state in the eye. In contrast, hydrolysis of cyclic nucleotides by cAMP and cGMP-specific phosphodiesterases (PDEs) produces the opposite of these and other effects mediated by increased cyclic nucleotide levels. PDEs appear to be particularly important in the regulation of cyclic nucleotides, considering the diversity found in this family of proteins. At least seven families of mammalian PDEs (PDE1-7) have been identified based on substrate specificity and affinity, sensitivity to cofactors, and sensitivity to inhibitory drugs (Beavo, J.A. (1995) Physiological Reviews 75:725-48). PDE inhibitors have been found to be particularly useful in treating various clinical disorders. Rolipram, a specific inhibitor of PDE4, has been used in the treatment of depression, and similar inhibitors are undergoing evaluation as anti-inflammatory agents. Theophylline is a nonspecific PDE inhibitor used in the treatment of bronchial asthma and other respiratory diseases (Banner, K.H. and Page, C.P. (1995) Eur. Respir. J. 8:996-1000).

Calcium Signaling Molecules

Ca^{+2} is another second messenger molecule that is even more widely used as an intracellular mediator than cAMP. Two pathways exist by which Ca^{+2} can enter the cytosol in response to extracellular signals: One pathway acts primarily in nerve signal transduction where Ca^{+2} enters a nerve terminal through a voltage-gated Ca^{+2} channel. The second is a more ubiquitous pathway in

which Ca^{+2} is released from the ER into the cytosol in response to binding of an extracellular signaling molecule to a receptor. Ca^{2+} directly activates regulatory enzymes, such as protein kinase C, which trigger signal transduction pathways. Ca^{2+} also binds to specific Ca^{2+}-binding proteins (CBPs) such as calmodulin (CaM) which then activate multiple target proteins in the cell including enzymes, membrane transport pumps, and ion channels. CaM interactions are involved in a multitude of cellular processes including, but not limited to, gene regulation, DNA synthesis, cell cycle progression, mitosis, cytokinesis, cytoskeletal organization, muscle contraction, signal transduction, ion homeostasis, exocytosis, and metabolic regulation (Celio, M.R. et al. (1996) Guidebook to Calcium-binding Proteins, Oxford University Press, Oxford, UK, pp. 15-20). Some Ca^{2+} binding proteins are characterized by the presence of one or more EF-hand Ca^{2+} binding motifs, which are comprised of 12 amino acids flanked by α-helices (Celio, supra). The regulation of CBPs has implications for the control of a variety of disorders. Calcineurin, a CaM-regulated protein phosphatase, is a target for inhibition by the immunosuppressive agents cyclosporin and FK506. This indicates the importance of calcineurin and CaM in the immune response and immune disorders (Schwaninger M. et al. (1993) J. Biol Chem. 268:23111-23115). The level of CaM is increased several-fold in tumors and tumor-derived cell lines for various types of cancer (Rasmussen, C.D. and Means, A.R. (1989) Trends in Neuroscience 12:433-438).

The annexins are a family of calcium-binding proteins that associate with the cell membrane (Towle, C.A. and Treadwell, B.V. (1992) J. Biol. Chem. 267:5416-23). Annexins reversibly bind to negatively charged phospholipids (phosphatidylcholine and phosphatidylserine) in a calcium dependent manner. Annexins participate in various processes pertaining to signal transduction at the plasma membrane, including membrane-cytoskeleton interactions, phospholipase inhibition, anticoagulation, and membrane fusion. Annexins contain four to eight repeated segments of about 60 residues. Each repeat folds into five alpha helices wound into a right-handed superhelix.

Signaling Complex Protein Domains

PDZ domains were named for three proteins in which this domain was initially discovered. These proteins include PSD-95 (postsynaptic density 95), Dlg (Drosophila lethal(1)discs large-1), and ZO-1 (zonula occludens-1). These proteins play important roles in neuronal synaptic transmission, tumor suppression, and cell junction formation, respectively. Since the discovery of these proteins, over sixty additional PDZ-containing proteins have been identified in diverse prokaryotic and eukaryotic organisms. This domain has been implicated in receptor and ion channel clustering and in the targeting of multiprotein signaling complexes to specialized functional regions of the cytosolic face of the plasma membrane. (For review of PDZ domain-containing proteins, see Ponting, C. P. et al. (1997) Bioessays 19:469-479.) A large proportion of PDZ domains are found in the eukaryotic MAGUK (membrane-associated guanylate kinase) protein family, members of which bind to the

intracellular domains of receptors and channels. However, PDZ domains are also found in diverse membrane-localized proteins such as protein tyrosine phosphatases, serine/threonine kinases, G-protein cofactors, and synapse-associated proteins such as syntrophins and neuronal nitric oxide synthase (nNOS). Generally, about one to three PDZ domains are found in a given protein, although up to nine PDZ domains have been identified in a single protein. The glutamate receptor interacting protein (GRIP) contains seven PDZ domains. GRIP is an adaptor that links certain glutamate receptors to other proteins and may be responsible for the clustering of these receptors at excitatory synapses in the brain (Dong, H. et al. (1997) Nature 386:279-284).

The SH3 domain is defined by homology to a region of the proto-oncogene c-Src, a cytoplasmic protein tyrosine kinase. SH3 is a small domain of 50 to 60 amino acids that interacts with proline-rich ligands. SH3 domains are found in a variety of eukaryotic proteins involved in signal transduction, cell polarization, and membrane-cytoskeleton interactions. In some cases, SH3 domain-containing proteins interact directly with receptor tyrosine kinases. For example, the SLAP-130 protein is a substrate of the T-cell receptor (TCR) stimulated protein kinase. SLAP-130 interacts via its SH3 domain with the protein SLP-76 to affect the TCR-induced expression of interleukin-2 (Musci, M.A. et al. (1997) J. Biol. Chem. 272:11674-11677). Another recently identified SH3 domain protein is macrophage actin-associated tyrosine-phosphorylated protein (MAYP) which is phosphorylated during the response of macrophages to colony stimulating factor-1 (CSF-1) and is likely to play a role in regulating the CSF-1-induced reorganization of the actin cytoskeleton (Yeung, Y.-G. et al. (1998) J. Biol. Chem. 273:30638-30642). The structure of SH3 is characterized by two antiparallel beta sheets packed against each other at right angles. This packing forms a hydrophobic pocket lined with residues that are highly conserved between different SH3 domains. This pocket makes critical hydrophobic contacts with proline residues in the ligand (Feng, S. et al. (1994) Science 266: 1241-47). Endophilin is an SH3 domain-containing protein implicated in synaptic vesicle endocytosis. (Micheva, K.D. (1997) 272:27239-27245).

A novel domain, called the WW domain, resembles the SH3 domain in its ability to bind proline-rich ligands. This domain was originally discovered in dystrophin, a cytoskeletal protein with direct involvement in Duchenne muscular dystrophy (Bork, P. and Sudol, M. (1994) Trends Biochem. Sci. 19:531-533). WW domains have since been discovered in a variety of intracellular signaling molecules involved in development, cell differentiation, and cell proliferation. The structure of the WW domain is composed of beta strands grouped around four conserved aromatic residues, generally tryptophan.

Like SH3, the SH2 domain is defined by homology to a region of c-Src. SH2 domains interact directly with phospho-tyrosine residues, thus providing an immediate mechanism for the regulation and transduction of receptor tyrosine kinase-mediated signaling pathways. For example, as

many as ten distinct SH2 domains are capable of binding to phosphorylated tyrosine residues in the activated PDGF receptor, thereby providing a highly coordinated and finely tuned response to ligand-mediated receptor activation. (Reviewed in Schaffhausen, B. (1995) Biochem. Biophys. Acta. 1242:61-75.)

Homer is a neuronal immediate early gene that is enriched at excitatory synapses (Xiao, B. et al. (1998) Neuron 21:707-716). Homer proteins form multivalent complexes that bind proline-rich motifs in group 1 metabotropic glutamate receptors and inositol triphosphate receptors, thereby coupling these receptors in a signaling complex (Tu, J.C. (1999) Neuron 23:583-592).

The pleckstrin homology (PH) domain was originally identified in pleckstrin, the predominant substrate for protein kinase C in platelets. Since its discovery, this domain has been identified in over 90 proteins involved in intracellular signaling or cytoskeletal organization. Proteins containing the pleckstrin homology domain include a variety of kinases, phospholipase-C isoforms, guanine nucleotide release factors, and GTPase activating proteins. For example, members of the FGD1 family contain both Rho-guanine nucleotide exchange factor (GEF) and PH domains, as well as a FYVE zinc finger domain. FGD1 is the gene responsible for faciogenital dysplasia, an inherited skeletal dysplasia (Pasteris, N.G. and Gorski, J.L. (1999) Genomics 60:57-66). Many PH domain proteins function in association with the plasma membrane, and this association appears to be mediated by the PH domain itself. PH domains share a common structure composed of two antiparallel beta sheets flanked by an amphipathic alpha helix. Variable loops connecting the component beta strands generally occur within a positively charged environment and may function as ligand binding sites (Lemmon, M. A. et al. (1996) Cell 85:621-624.). n-Chimaerin is a GAP involved in the formation of lamellipodia and filopodia in neuroblastoma cells. (Kozma, R. et al. (1996) Mol. Cell Biol. 16:5069-5080.)

Ankyrin (ANK) repeats mediate protein-protein interactions associated with diverse intracellular signaling functions. For example, ANK repeats are found in proteins involved in cell proliferation such as kinases, kinase inhibitors, tumor suppressors, and cell cycle control proteins. (See, for example, Kalus, W. et al. (1997) FEBS Lett. 401:127-132; Ferrante, A. W. et al. (1995) Proc. Natl. Acad. Sci. USA 92:1911-1915.) These proteins generally contain multiple ANK repeats, each composed of about 33 amino acids. Myotrophin is an ANK repeat protein that plays a key role in the development of cardiac hypertrophy, a contributing factor to many heart diseases. Structural studies show that the myotrophin ANK repeats, like other ANK repeats, each form a helix-turn-helix core preceded by a protruding "tip." These tips are of variable sequence and may play a role in protein-protein interactions. The helix-turn-helix region of the ANK repeats stack on top of one another and are stabilized by hydrophobic interactions (Yang, Y. et al. (1998) Structure 6:619-626).

The tetratrico peptide repeat (TPR) is a 34 amino acid repeated motif found in organisms

from bacteria to humans. TPRs are predicted to form ampipathic helices, and appear to mediate protein-protein interactions. TPR domains are found in CDC16, CDC23, and CDC27, members the the anaphase promoting complex which targets proteins for degradation at the onset of anaphase. Other processes involving TPR proteins include cell cycle control, transcription repression, stress response, and protein kinase inhibition. (Lamb, J.R. et al. (1995) Trends Biochem. Sci. 20:257-259.)

The armadillo/beta-catenin repeat is a 42 amino acid motif which forms a superhelix of alpha helices when tandemly repeated. The structure of the armadillo repeat region from beta-catenin revealed a shallow groove of positive charge on one face of the superhelix, which is a potential binding surface. The armadillo repeats of beta-catenin, plakoglobin, and p120^{cas} bind the cytoplasmic domains of cadherins. Beta-catenin/cadherin complexes are targets of regulatory signals that govern cell adhesion and mobility. (Huber, A.H. et al. (1997) Cell 90:871-882.)

The discovery of new intracellular signaling proteins and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cell proliferative, autoimmune/inflammatory, reproductive, and developmental disorders.

SUMMARY OF THE INVENTION

The invention features purified polypeptides, intracellular signaling molecules, referred to collectively as "INTRA" and individually as "INTRA-1," "INTRA-2," "INTRA-3," "INTRA-4," "INTRA-5," "INTRA-6," "INTRA-7," "INTRA-8," "INTRA-9," "INTRA-10," "INTRA-11," "INTRA-12," "INTRA-13," "INTRA-14," "INTRA-15," "INTRA-16," "INTRA-17," "INTRA-18," "INTRA-19," "INTRA-20," "INTRA-21," "INTRA-22," "INTRA-23," "INTRA-24," "INTRA-25," "INTRA-26," "INTRA-27," "INTRA-28," "INTRA-29," "INTRA-30," "INTRA-31," "INTRA-32," "INTRA-33," "INTRA-34," "INTRA-35," "INTRA-36," "INTRA-37," "INTRA-38," "INTRA-39," "INTRA-40," "INTRA-41," "INTRA-42," "INTRA-43," "INTRA-44," "INTRA-45," "INTRA-46," "INTRA-47," "INTRA-48," "INTRA-49," "INTRA-50," "INTRA-51," and "INTRA-52." In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-52.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising

an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-52. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:53-104.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52.

The invention further provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:53-104, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:53-104, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:53-104, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:53-104, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:53-104, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:53-104, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention further provides a pharmaceutical composition comprising an effective amount of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence

selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and a pharmaceutically acceptable excipient. In one embodiment, the pharmaceutical composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional INTRA, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional INTRA, comprising administering to a patient in need of such treatment the pharmaceutical composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional INTRA, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention further provides a method of screening for a compound that specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an

amino acid sequence selected from the group consisting of SEQ ID NO:1-52. b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-52. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:53-104, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

BRIEF DESCRIPTION OF THE TABLES

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding INTRA.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of INTRA.

Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases,

disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding INTRA were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"INTRA" refers to the amino acid sequences of substantially purified INTRA obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of INTRA. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of INTRA either by directly interacting with INTRA or by acting on components of the biological pathway in which INTRA participates.

An "allelic variant" is an alternative form of the gene encoding INTRA. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding INTRA include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as INTRA or a polypeptide with at least one functional characteristic of INTRA. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding INTRA, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding INTRA. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent INTRA. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of INTRA is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of INTRA. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of INTRA either by directly interacting with INTRA or by acting on components of the biological pathway in which

INTRA participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, $F(ab')_2$, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind INTRA polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic INTRA, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding INTRA or fragments of INTRA may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (PE Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser
Gln	Asn, Glu, His
Glu	Asp, Gln, His
Gly	Ala
His	Asn, Arg, Gln, Glu
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	His, Met, Leu, Trp, Tyr
Ser	Cys, Thr
Thr	Ser, Val
Trp	Phe, Tyr
Tyr	His, Phe, Trp
Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide

backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to a chemically modified polynucleotide or polypeptide. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

A "fragment" is a unique portion of INTRA or the polynucleotide encoding INTRA which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:53-104 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:53-104, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:53-104 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:53-104 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:53-104 and the region of SEQ ID NO:53-104 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-52 is encoded by a fragment of SEQ ID NO:53-104. A fragment of SEQ ID NO:1-52 comprises a region of unique amino acid sequence that specifically

identifies SEQ ID NO:1-52. For example, a fragment of SEQ ID NO:1-52 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-52. The precise length of a fragment of SEQ ID NO:1-52 and the region of SEQ ID NO:1-52 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A "full-length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full-length" polynucleotide sequence encodes a "full-length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at http://www.ncbi.nlm.nih.gov/BLAST/. The BLAST software suite includes various sequence analysis programs including "blastn." that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to

compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise

comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (Apr-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for chromosome replication, segregation and maintenance.

The term "humanized antibody" refers to an antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 μg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature

under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 μg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of INTRA which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of INTRA which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of INTRA. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of INTRA.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Post-translational modification" of an INTRA may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of INTRA.

"Probe" refers to nucleic acid sequences encoding INTRA, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also

be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al.,1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence

that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be use to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs). Regulatory elements interact with host or viral proteins which control transcription, translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding INTRA, or fragments thereof, or INTRA itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are

removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants, and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of

the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human intracellular signaling molecules (INTRA), the polynucleotides encoding INTRA, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative, autoimmune/inflammatory, neurological, gastrointestinal, reproductive, and developmental disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding INTRA. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each INTRA were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. In some cases, GenBank sequence identifiers are also shown in column 5. The Incyte clones and GenBank cDNA sequences, where indicated, in column 5 were used to assemble the consensus nucleotide sequence of each INTRA and are useful as fragments in

hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis along with relevant citations, all of which are expressly incorporated by reference herein in their entirety; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding INTRA. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:53-104 and to distinguish between SEQ ID NO:53-104 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express INTRA as a fraction of total tissues expressing INTRA. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing INTRA as a fraction of total tissues expressing INTRA. Column 5 lists the vectors used to subclone each cDNA library. Of particular interest is the expression of SEQ ID NO:88 and SEQ ID NO:94 in reproductive tissues, of SEQ ID NO:99, SEQ ID NO:100, and SEQ ID NO:103 in hematopoietic/immune tissues, and of SEQ ID NO:96 in cardiovascular tissues.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding INTRA were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO:58 maps to chromosome 7 within the interval from 84.40 to 90.30 centiMorgans. This interval also contains an EST with high similarity to thyroid disease hypothetical autoantigen. SEQ ID NO:67 maps to chromosome 16 within the interval from 119.20 centiMorgans to q-terminus. This interval also contains the paraplegin gene, mutations in which cause spastic paraplegia and OXPHOS impairment. SEQ ID NO:70 maps to chromosome 11 within the interval from 59.50 to 62.50 centiMorgans. SEQ ID NO:71 maps to chromosome 7 within the interval from 138.0 to 145.8 centiMorgans. SEQ ID NO:73 maps to chromosome 12 within the interval from 76.5 to 84.2 centiMorgans. SEQ ID NO:77 maps to chromosome 7 within the interval from 4.8 to 10.6 centiMorgans and to chromosome 4 within the interval from 56.7 to 60.5 centiMorgans. The interval

on chromosome 7 from from 4.8 to 10.6 centiMorgans also contains a gene associated with cell proliferation. The interval on chromosome 4 from 56.7 to 60.5 centiMorgans also contains a gene associated with cell proliferation. SEQ ID NO:79 maps to chromosome 15 within the interval from 32.2 to 47.1 centiMorgans. This interval also contains a gene associated with cell proliferation. SEQ ID NO:80 maps to chromosome 20 within the interval from 50.2 to 53.6 centiMorgans. This interval also contains a gene associated with cell differentiation. SEQ ID NO:84 maps to chromosome 3 within the interval from 142.2 to 148.7 centiMorgans. SEQ ID NO:87 maps to chromosome 5 within the interval from 141.4 to 147.1 centiMorgans. SEQ ID NO:91 maps to chromosome 12 within the interval from 62.7 to 67.3 centiMorgans. SEQ ID NO:95 maps to chromosome 15 within the interval from 45.5 to 58.8 centiMorgans. SEQ ID NO:97 maps to the X chromosome within the interval from 112.8 to 139.4 centiMorgans.

The invention also encompasses INTRA variants. A preferred INTRA variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the INTRA amino acid sequence, and which contains at least one functional or structural characteristic of INTRA.

The invention also encompasses polynucleotides which encode INTRA. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:53-104, which encodes INTRA. The polynucleotide sequences of SEQ ID NO:53-104, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding INTRA. In particular, such a variant polynucleotide sequence will have at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding INTRA. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:53-104 which has at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:53-104. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of INTRA.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding INTRA, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These

combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring INTRA, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode INTRA and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring INTRA under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding INTRA or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding INTRA and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode INTRA and INTRA derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding INTRA or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:53-104 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (PE Biosystems, Foster City CA), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (PE Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (PE Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting

sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding INTRA may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate

software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, PE Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode INTRA may be cloned in recombinant DNA molecules that direct expression of INTRA, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express INTRA.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter INTRA-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Crameri, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of INTRA, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding INTRA may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.)

Alternatively, INTRA itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (PE Biosystems). Additionally, the amino acid sequence of INTRA, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a sequence of a naturally occurring polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, supra, pp. 28-53.)

In order to express a biologically active INTRA, the nucleotide sequences encoding INTRA or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding INTRA. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding INTRA. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding INTRA and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding INTRA and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding INTRA. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; Scorer, C.A. et al. (1994) Bio/Technology 12:181-184; Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945; Takamatsu, N. (1987) EMBO J. 6:307-311; Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659; and Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) Cancer Gen. Ther. 5(6):350-356; Yu, M. et al., (1993) Proc. Natl. Acad. Sci. USA 90(13):6340-6344; Buller, R.M. et al. (1985) Nature 317(6040):813-815; McGregor, D.P. et al. (1994) Mol. Immunol. 31(3):219-226; and Verma, I.M. and N. Somia (1997) Nature 389:239-242.) The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding INTRA. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding INTRA can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding INTRA into the vector's multiple cloning site disrupts the *lac*Z gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of INTRA are needed, e.g. for the production of antibodies, vectors which direct high level expression of INTRA may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of INTRA. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH

promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, supra; and Scorer, supra.)

Plant systems may also be used for expression of INTRA. Transcription of sequences encoding INTRA may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, supra; Broglie, supra; and Winter, supra.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding INTRA may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses INTRA in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of INTRA in cell lines is preferred. For example, sequences encoding INTRA can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*⁻ and *apr*⁻ cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), ß glucuronidase and its substrate ß-glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding INTRA is inserted within a marker gene sequence, transformed cells containing sequences encoding INTRA can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding INTRA under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding INTRA and that express INTRA may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of INTRA using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on INTRA is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and

Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding INTRA include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding INTRA, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding INTRA may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode INTRA may be designed to contain signal sequences which direct secretion of INTRA through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding INTRA may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric INTRA protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of INTRA activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available

affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the INTRA encoding sequence and the heterologous protein sequence, so that INTRA may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled INTRA may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ^{35}S-methionine.

INTRA of the present invention or fragments thereof may be used to screen for compounds that specifically bind to INTRA. At least one and up to a plurality of test compounds may be screened for specific binding to INTRA. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

In one embodiment, the compound thus identified is closely related to the natural ligand of INTRA, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which INTRA binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express INTRA, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing INTRA or cell membrane fractions which contain INTRA are then contacted with a test compound and binding, stimulation, or inhibition of activity of either INTRA or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with INTRA, either in

solution or affixed to a solid support, and detecting the binding of INTRA to the compound. Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

INTRA of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of INTRA. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for INTRA activity, wherein INTRA is combined with at least one test compound, and the activity of INTRA in the presence of a test compound is compared with the activity of INTRA in the absence of the test compound. A change in the activity of INTRA in the presence of the test compound is indicative of a compound that modulates the activity of INTRA. Alternatively, a test compound is combined with an in vitro or cell-free system comprising INTRA under conditions suitable for INTRA activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of INTRA may do so indirectly and need not come in direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding INTRA or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent No. 5,175,383 and U.S. Patent No. 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding INTRA may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate

into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding INTRA can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding INTRA is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress INTRA, e.g., by secreting INTRA in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of INTRA and intracellular signaling molecules. In addition, the expression of INTRA is closely associated with cancers of the hematopoetic/immune, nervous, gastrointestinal, and reproductive, systems therefore, INTRA appears to play a role in cell proliferative, autoimmune/inflammatory, neurological, gastrointestinal, reproductive, and developmental disorders. In the treatment of disorders associated with increased INTRA expression or activity, it is desirable to decrease the expression or activity of INTRA. In the treatment of disorders associated with decreased INTRA expression or activity, it is desirable to increase the expression or activity of INTRA.

Therefore, in one embodiment, INTRA or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of INTRA. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, hematopoietic cancer including lymphoma, leukemia, and myeloma; and other cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, adenoma, carcinoma and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's

disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma ; and a gastrointestinal disorder such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha$_1$-antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and a hepatic tumor including a nodular hyperplasia,.a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system

disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; and a gastrointestinal disorder such as esophagitis, esophageal carcinoma, gastritis, gastric carcinoma, inflammatory bowel disease, cholecystitis, infections of the intestinal tract, pancreatitis, pancreatic carcinoma, cirrhosis, hepatitis, hepatoma, colitis, colonic carcinoma, and Crohn's disease.

In another embodiment, a vector capable of expressing INTRA or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of INTRA including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified INTRA in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of INTRA including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of INTRA may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of INTRA including, but not limited to, those listed above.

In a further embodiment, an antagonist of INTRA may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of INTRA. Examples of such disorders include, but are not limited to, those cell proliferative, autoimmune/inflammatory, neurological, gastrointestinal, reproductive, and developmental disorders described above. In one aspect, an antibody which specifically binds INTRA may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express INTRA.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding INTRA may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of INTRA including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of INTRA may be produced using methods which are generally known in the art. In particular, purified INTRA may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind INTRA. Antibodies to INTRA may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with INTRA or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to INTRA have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of INTRA amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to INTRA may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce INTRA-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for INTRA may also be generated. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between INTRA and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering INTRA epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for INTRA. Affinity is expressed as an association constant, K_a, which is defined as the molar concentration of INTRA-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple INTRA epitopes, represents the average affinity, or avidity, of the antibodies for INTRA. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular INTRA epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the INTRA-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of INTRA, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml,

preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of INTRA-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al., supra.)

In another embodiment of the invention, the polynucleotides encoding INTRA, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding INTRA. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding INTRA. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) J. Allergy Clin. Immunol. 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) Blood 76:271; Ausubel, supra; Uckert, W. and W. Walther (1994) Pharmacol. Ther. 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) Br. Med. Bull. 51(1):217-225; Boado, R.J. et al. (1998) J. Pharm. Sci. 87(11):1308-1315; and Morris, M.C. et al. (1997) Nucleic Acids Res. 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding INTRA may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) Science 270:404-410; Verma, I.M. and Somia, N. (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated

cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in INTRA expression or regulation causes disease, the expression of INTRA from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in INTRA are treated by constructing mammalian expression vectors encoding INTRA and introducing these vectors by mechanical means into INTRA-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J-L. and H. Récipon (1998) Curr. Opin. Biotechnol. 9:445-450).

Expression vectors that may be effective for the expression of INTRA include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). INTRA may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or β-actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) Proc. Natl. Acad. Sci. U.S.A. 89:5547-5551; Gossen, M. et al. (1995) Science 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) Curr. Opin. Biotechnol. 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and H.M. Blau, supra)), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding INTRA from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) Virology 52:456-467), or by electroporation (Neumann, E. et al.

(1982) EMBO J. 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to INTRA expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding INTRA under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) Proc. Natl. Acad. Sci. U.S.A. 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) J. Virol. 61:1639-1646; Adam, M.A. and A.D. Miller (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R. et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., $CD4^+$ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. U.S.A. 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding INTRA to cells which have one or more genetic abnormalities with respect to the expression of INTRA. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu. Rev. Nutr. 19:511-544; and Verma, I.M. and N. Somia (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding INTRA to target cells which have one or more genetic abnormalities with

respect to the expression of INTRA. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing INTRA to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) Exp. Eye Res.169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) J. Virol. 73:519-532 and Xu, H. et al. (1994) Dev. Biol. 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding INTRA to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) Curr. Opin. Biotech. 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full-length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for INTRA into the alphavirus genome in place of the capsid-coding region results in the production of a large number of INTRA-coding RNAs and the synthesis of high levels of INTRA in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) Virology 228:74-83). The wide host range of alphaviruses will allow the introduction of INTRA into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA

transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding INTRA.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding INTRA. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs

and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding INTRA. Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased INTRA expression or activity, a compound which specifically inhibits expression of the polynucleotide encoding INTRA may be therapeutically useful, and in the treament of disorders associated with decreased INTRA expression or activity, a compound which specifically promotes expression of the polynucleotide encoding INTRA may be therapeutically useful.

At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a polynucleotide encoding INTRA is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding INTRA are assayed by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence complementary to the sequence of the polynucleotide encoding INTRA. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound effective in altering expression of a specific polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids

Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruice, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruice, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such pharmaceutical compositions may consist of INTRA, antibodies to INTRA, and mimetics, agonists, antagonists, or inhibitors of INTRA.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Pharmaceutical compositions for pulmonary administration may be prepared in liquid or dry powder form. These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton, J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

Specialized forms of pharmaceutical compositions may be prepared for direct intracellular delivery of macromolecules comprising INTRA or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, INTRA or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example INTRA or fragments thereof, antibodies of INTRA, and agonists, antagonists or inhibitors of INTRA, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD_{50}/ED_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind INTRA may be used for the diagnosis of disorders characterized by expression of INTRA, or in assays to monitor patients being treated with INTRA or agonists, antagonists, or inhibitors of INTRA. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for INTRA include methods which utilize the antibody and a label to detect INTRA in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring INTRA, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of INTRA expression. Normal or standard values for INTRA expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to INTRA under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of INTRA expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding INTRA may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of INTRA may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of INTRA, and to monitor regulation of INTRA levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding INTRA or closely related molecules may be used to identify nucleic acid sequences which encode INTRA. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the

probe identifies only naturally occurring sequences encoding INTRA, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the INTRA encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:53-104 or from genomic sequences including promoters, enhancers, and introns of the INTRA gene.

Means for producing specific hybridization probes for DNAs encoding INTRA include the cloning of polynucleotide sequences encoding INTRA or INTRA derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding INTRA may be used for the diagnosis of disorders associated with expression of INTRA. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, hematopoietic cancer including lymphoma, leukemia, and myeloma; and other cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, adenoma, carcinoma and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal

circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma ; and a gastrointestinal disorder such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha$_1$-antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and a hepatic tumor including a nodular hyperplasia,.a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; and a gastrointestinal disorder such as esophagitis, esophageal carcinoma, gastritis, gastric carcinoma, inflammatory bowel disease, cholecystitis, infections of the intestinal tract, pancreatitis, pancreatic carcinoma, cirrhosis, hepatitis, hepatoma, colitis, colonic carcinoma, and Crohn's disease. The polynucleotide sequences encoding INTRA may be used in Southern or northern analysis, dot blot, or other membrane-based

technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered INTRA expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding INTRA may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding INTRA may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding INTRA in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of INTRA, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding INTRA, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding INTRA may involve the use of PCR. These oligomers may be chemically synthesized, generated

enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding INTRA, or a fragment of a polynucleotide complementary to the polynucleotide encoding INTRA, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding INTRA may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from the polynucleotide sequences encoding INTRA are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSCCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed in silico SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of INTRA include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described in Seilhamer, J.J. et al., "Comparative Gene Transcript Analysis," U.S. Patent No. 5,840,484, incorporated herein by reference. The microarray may also be

used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, antibodies specific for INTRA, or INTRA or fragments thereof may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described above.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding INTRA may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, e.g., Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man

(OMIM) World Wide Web site. Correlation between the location of the gene encoding INTRA on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, INTRA, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between INTRA and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with INTRA, or fragments thereof, and washed. Bound INTRA is then detected by methods well known in the art. Purified INTRA can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding INTRA specifically compete with a test compound for binding INTRA. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with INTRA.

In additional embodiments, the nucleotide sequences which encode INTRA may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/139,566 (filing date 16 June 1999), U.S. Ser. No. 60/149,640 (filing date 17 August 1999), and U.S. Ser. No. 60/164,417 (filing date 9 November 1999), are hereby expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERSCRIPT plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Genomics, Palo Alto CA). Recombinant

plasmids were transformed into competent E. coli cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5α, DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids obtained as described in Example I were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (PE Biosystems) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (PE Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VI.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools,

programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:53-104. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is

much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score x Percent Identity}}{5 \times \text{minimum } \{\text{length(Seq. 1)}, \text{length(Seq. 2)}\}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding INTRA occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

V. Chromosomal Mapping of ABBR Encoding Polynucleotides

The cDNA sequences which were used to assemble SEQ ID NO:8-14 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:8-14 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for

Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO:, to that map location.

The genetic map locations of SEQ ID NO:8-14 [fill in the specific SEQ ID NOs if not all of the sequences have been mapped] are described in The Invention as ranges, or intervals, of human chromosomes. [Include the following sentence if any of your sequences have more than one map location.] More than one map location is reported for SEQ ID NO:8-14 [fill in specific SEQ ID NO:s], indicating that previously mapped sequences having similarity, but not complete identity, to SEQ ID NO:8-14 [fill in specific SEQ ID NO:s] were assembled into their respective clusters. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Human genome maps and other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site (http://www.ncbi.nlm.nih.gov/genemap/), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

VI. Extension of INTRA Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:53-104 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+}, $(NH_4)_2SO_4$, and β-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C,

2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 μl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 μl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μl to 10 μl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent E. coli cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethysulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems).

In like manner, the polynucleotide sequences of SEQ ID NO:53-104 are used to obtain 5' regulatory sequences using the procedure above, along with oligonucleotides designed for such extension, and an appropriate genomic library.

VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:53-104 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

VIII. Microarrays

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, supra), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned technologies should be uniform and solid with a non-porous surface (Schena (1999), supra). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645; Marshall, A. and J. Hodgson (1998) Nat. Biotechnol. 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a

fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser desorbtion and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)$^+$ RNA is purified using the oligo-(dT) cellulose method. Each poly(A)$^+$ RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/μl oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/μl RNase inhibitor, 500 μM dATP, 500 μM dGTP, 500 μM dTTP, 40 μM dCTP, 40 μM dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)$^+$ RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)$^+$ RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA. After incubation at 37 °C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85 °C to the stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 μl 5X SSC/0.2% SDS.

Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5 μg. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1 μl of the array element DNA, at an average concentration of 100 ng/μl, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60 °C followed by washes in 0.2% SDS and distilled water as before.

Hybridization

Hybridization reactions contain 9 μl of sample mixture consisting of 0.2 μg each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample mixture is heated to 65 °C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm^2 coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 μl of 5X SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60 °C. The arrays are washed for 10 min at 45 °C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45 °C in a second wash buffer (0.1X SSC), and dried.

Detection

Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

IX. Complementary Polynucleotides

Sequences complementary to the INTRA-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring INTRA. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of INTRA. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the INTRA-encoding transcript.

X. Expression of INTRA

Expression and purification of INTRA is achieved using bacterial or virus-based expression systems. For expression of INTRA in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory

element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express INTRA upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of INTRA in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding INTRA by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, INTRA is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from INTRA at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified INTRA obtained by these methods can be used directly in the assays shown in Examples XI, XII, and XV.

XI. Demonstration of INTRA Activity

INTRA activity is associated with its ability to form protein-protein complexes and is measured by its ability to regulate growth characteristics of NIH3T3 mouse fibroblast cells. A cDNA encoding INTRA is subcloned into an appropriate eukaryotic expression vector. This vector is transfected into NIH3T3 cells using methods known in the art. Transfected cells are compared with non-transfected cells for the following quantifiable properties: growth in culture to high density, reduced attachment of cells to the substrate, altered cell morphology, and ability to induce tumors when injected into immunodeficient mice. The activity of INTRA is proportional to the extent of increased growth or frequency of altered cell morphology in NIH3T3 cells transfected with INTRA.

Alternatively, INTRA activity is measured by binding of INTRA to radiolabeled formin polypeptides containing the proline-rich region that specifically binds to SH3 containing proteins

(Chan, D.C. et al. (1996) EMBO J. 15: 1045-54). Samples of INTRA are run on SDS-PAGE gels, and transferred onto nitrocellulose by electroblotting. The blots are blocked for 1 hr at room temperature in TBST (137 mM NaCl, 2.7 mM Kcl, 25 mM Tris (pH 8.0) and 0.1% Tween-20) containing non-fat dry milk. Blots are then incubated with TBST containing the radioactive formin polypeptide for 4 hrs to overnight. After washing the blots four times with TBST, the blots are exposed to autoradiographic film. Radioactivity is quantitated by cutting out the radioactive spots and counting them in a radioisotope counter. The amount of radioactivity recovered is proportional to the activity of INTRA in the assay.

Alternatively, INTRA activity is demonstrated by measuring the binding of INTRA to Ca^{2+} using a Ca^{2+} overlay system (Weis, K. et al. (1994) J. Biol. Chem. 269:19142-19150). Purified INTRA is transferred and immobilized onto a nitrocellulose membrane. The membrane is washed three times with buffer (60 mM KCl, 5 mM $MgCl_2$, 10 mM imidazole-HCl, pH 6.8) and incubated in this buffer for 10 minutes with 1 μCi [$^{45}Ca^{2+}$] (NEN-DuPont, Boston, MA). Unbound [$^{45}Ca^{2+}$] is removed from the membrane by washing with water, and the membrane is dried. Membrane-bound [$^{45}Ca^{2+}$] is detected by autoradiography and quantified using image analysis systems and software. INTRA activity is proportional to the amount of [$^{45}Ca^{2+}$] detected on the membrane.

Alternatively, INTRA activity is assayed by measuring the conversion of ^{3}H-cAMP to ^{3}H-adenosine in the presence of INTRA and 5' nucleotidase. INTRA is added to a solution containing 50 mM Tris-HCl pH 7.5, 10 mM $MgCl^2$, 0.1 unit 5'nucleotidase (from Crotalus atrox venom), and 0.0064-2.0 uM ^{3}H- cAMP and the reaction is incubated at 37°C for a time period that would yield less than 15% cAMP hydrolysis in order to avoid non-linearity associated with product inhibition. Soluble radioactivity associated with ^{3}H-adenosine is quantitated using a Beta scintillation counter. The amount of radioactivity recovered is proportional to the activity of INTRA in the reaction.

XII. Functional Assays

INTRA function is assessed by expressing the sequences encoding INTRA at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10 μg of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP;

Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of INTRA on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding INTRA and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding INTRA and other genes of interest can be analyzed by northern analysis or microarray techniques.

XIII. Production of INTRA Specific Antibodies

INTRA substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the INTRA amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (PE Biosystems) using FMOC chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-INTRA activity by, for example, binding the peptide or INTRA to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIV. Purification of Naturally Occurring INTRA Using Specific Antib dies

Naturally occurring or recombinant INTRA is substantially purified by immunoaffinity chromatography using antibodies specific for INTRA. An immunoaffinity column is constructed by covalently coupling anti-INTRA antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing INTRA are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of INTRA (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/INTRA binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and INTRA is collected.

XV. Identification of Molecules Which Interact with INTRA

INTRA, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled INTRA, washed, and any wells with labeled INTRA complex are assayed. Data obtained using different concentrations of INTRA are used to calculate values for the number, affinity, and association of INTRA with the candidate molecules.

Alternatively, molecules interacting with INTRA are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989, Nature 340:245-246), or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

INTRA may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 6,057,101).

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	53	129042	TESTNOT01	129042H1 (TESTNOT01), 129042T6 (TESTNOT01), 594163H1 (BRAVUNT02), 1376353T6 (LUNGNOT10), 1968641R6 (BRSTNOT04), 4193335F6 (BRAPDIT01), 5636985H1 (UTRSTMR01)
2	54	778003	COLNNOT05	778003H1 (COLNNOT05), 778003X29 (COLNNOT05), 793138X17 (PROSTUT03), 5533562H1 (HEARFET05)
3	55	1418671	KIDNNOT09	458013F1 (KERANOT01), 461367R6 (KERANOT01), 1418671H1 (KIDNNOT09), 1418671X301D1 (KIDNNOT09), 1452670F1 (PENITUT01), 1455886F1 (COLNFET02), 2921431H1 (SININOT04)
4	56	1456841	COLNFET02	214180X3 (STOMNOT01), 1456841H1 (COLNFET02), 1517021F1 (PANCTUT01), 2280709F6 (COLSUCT01), SBFA01757F1, SBFA04860F1, SBFA03431F1
5	57	2020010	CONNNOT01	520251R1 (MMLR2DT01), 552501H1 (SCORNOT01), 1297508H1 (BRSTNOT07), 1417085H1 (BRAINOT12), 1455946F1 (COLNFET02), 1864670H1 (PROSNOT19), 1922941R6 (BRSTTUT01), 1922941T6 (BRSTTUT01), 1930785H1 (COLNTUT03), 2020010F6 (CONNNOT01), 2020010H1 (CONNNOT01), 2879789H1 (UTRSTUT05), 3324110H1 (PTHYNOT03), 3766286H1 (BRSTNOT24), 4305754H1 (TESTTUT03)
6	58	2149037	BRAINOT09	1382860F1 (BRAITUT08), 1709135F6 (PROSNOT16), 1758155R6 (PITUNOT03), 1861076F6 (PROSNOT19), 2149037H1 (BRAINOT09), 2149037X15F1 (BRAINOT09), 2280366H1 (PROSNON01), 2524642F6 (BRAITUT21), 2590271H1 (LUNGNOT22), 2970418H2 (HEAONOT02), 3084127H1 (BRAIFET01), 4789892T6 (EPIBUNT01)
7	59	2162179	ENDCNOT02	2162179F6 (ENDCNOT02), 2162179H1 (ENDCNOT02), 3865236H1 (BRAITUT07)
8	60	2244706	HIPONON02	2244706H1 (HIPONON02), 3272168F6 (BRAINOT20), SBWA00950V1, SBWA03641V1, SBWA02322V1

Table 1 (cont.)

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
9	61	2316805	OVARNOT02	363271R6 (PROSNOT01), 855363H1 (NGANNOT01), 1209030T1 (BRSTNOT02), 1265148R1 (SYNORAT05), 1294807F1 (PGANNOT03), 1351585F1 (LATRTUT02), 1852006F6 (LUNGFET03), 2316805H1 (OVARNOT02), 2320867H1 (OVARNOT02), 3563231F6 (SKINNOT05)
10	62	2320010	OVARNOT02	448783H1 (TLYMNOT02), 470134R1 (MMLR1DT01), 720124F1 (SYNOOAT01), 1873477F6 (LEUKNOT02), 2320010H1 (OVARNOT02), 3049510T6 (LUNGNOT25), 3087109F6 (HEAONOT03), 4144881H1 (SINITUT04), 5089346H1 (UTRSTMR01)
11	63	2564901	ADRETUT01	214410F1 (STOMNOT01), 927356R1 (BRAINOT04), 2564901H1 (ADRETUT01)
12	64	2615168	GBLANOT01	1445950F6 (PLACNOT02), 2615168H1 (GBLANOT01), 2746963F6 (LUNGTUT11), 2746963T6 (LUNGTUT11), 3250984H1 (SEMVNOT03), 3459378H1 (293TF1T01), 3831615H1 (PANCNOT17), 4334378H1 (KIDCTMT01), 4818908H1 (PROSTUT17)
13	65	2658329	LUNGTUT09	1210539H1 (BRSTNOT02), 1210539R6 (BRSTNOT02), 1985147R6.comp (LUNGAST01), 2311120R6 (NGANNOT01), 2658329H1 (LUNGTUT09), 2717243F6 (THYRNOT09), 2831384F7 (TLYMNOT03), 3846358H1 (DENDNOT01), 4898171H1 (OVARDIT01)
14	66	2708944	PONSAZT01	309840R6 (TMLR2DT01), 1241166R6 (LUNGNOT03), 1381850H1 (BRAITUT08), 2194624F6 (THYRTUT03), 2212407F6 (SINTFET03), 2708944F6 (PONSAZT01), 2708944H1 (PONSAZT01), 4895659H1 (LIVRTUT12)
15	67	3315012	293TF1T01	532568R6 (BRAINOT03), 1300242F1 (BRSTNOT07), 1329265F1 (PANCNOT07), 1439786H1 (PANCNOT08), 2327916X23C1 (COLNNOT11), 2381037X37C1 (ISLTNOT01), 2381037X39C1 (ISLTNOT01), 3315012H1 (293TF1T01), SAEB00241R1
16	68	4155412	ADRENOT14	555524R6 (SCORNOT01), 4155412F6 (ADRENOT14), 4155412H1 (ADRENOT14), 4943387F6 (BRAIFEN05)

Table 1 (cont.)

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
17	69	4831840	BRAVTXT03	286660H1 (EOSIHET02), 422026H1 (CARCTXT01), 1734445F6 (COLNNOT22), 1734445T6 (COLNNOT22), 1970421F6 (UCMCL5T01), 2512308H1 (CONUTUT01), 4831840H1 (BRAVTXT03)
18	70	5676581	293TF2T01	702633R6 (SYNORAT03), 1000026R1 (BRSTNOT03), 2631308F6 (COLNTUT15), 3012653H1 (MUSCNOT07), 3252744H1 (OVARTUN01), 3315168H2 (293TF2T01), 3530354H1 (BLADNOT09), 4289137H1 (BRABDIR01), 4974749H1 (HELATXT03), 5676581H1 (293TF2T01)
19	71	034159	THP1NOB01	034159H1 (THP1NOB01), 034159X305D3 (THP1NOB01), 406358R6 (EOSIHET02), 1974550F6 (UCMCL5T01), 3471911H1 (LUNGNOT27), 3522363H1 (ESOGTUN01), 4326520H1 (TLYMUNT01), SCJA01020V1, SCIA01764V1
20	72	129023	TESTNOT01	129023R6 (TESTNOT01), 775480R1 (COLNNOT05), 1649938F6 (PROSTUT09), 2518140F6 (BRAITUT21), 2688123H1 (LUNGNOT23), 4306520H1 (MONOTXT01)
21	73	1358940	LUNGNOT09	879273R1 (THYRNOT02), 967670T1 (BRSTNOT05), 1358940F6 (LUNGNOT09), 1358940H1 (LUNGNOT09), 1809259H1 (PROSTUT12), 1818790F6 (PROSNOT20), 1886716F6 (BLADTUT07), 1905126F6 (OVARNOT07), 3508881H1 (CONCNOT01), 3687018F6 (HEAANOT01), 3812474F6 (TONSNOT03)
22	74	1682320	PROSNOT15	1214001T1 (BRSTTUT01), 1259957F1 (MENITUT03), 1375132H1 (LUNGNOT10), 1682320H1 (PROSNOT15), 3137047H1 (SMCCNOT01), 3805984H1 (BLADTUT03), 3806302H1 (BLADTUT03)
23	75	1728263	PROSNOT14	1269315H1 (BRAINOT09), 1453910F1 (PENITUT01), 1728263H1 (PROSNOT14), g2115530
24	76	1867626	SKINBIT01	667711T6 (SCORNOT01), SXYA01116V1, SXYA01833V1, SXYA02442V1

Table 1 (cont.)

25	77	1990126	CORPNOT02	426763T6 (BLADNOT01), 1647316F6 (PROSTUT09), 1757430R6 (PITUNOT03), 1830621F6 (THP1AZT01), 1990126H1 (CORPNOT02), 3250740H1 (SEMVNOT03)
26	78	2104180	BRAITUT02	1350750F1 (LATRTUT02), 1502445F1 (BRAITUT07), 1519125X301D1 (BLADTUT04), 2104180H1 (BRAITUT02), 2733677H1 (OVARTUT04)
27	79	2122241	BRSTNOT07	1402761H1 (LATRTUT02), 1402761T6 (LATRTUT02), 2122241F6 (BRSTNOT07), 2122241H1 (BRSTNOT07), 4989861H1 (LIVRTUT11)
28	80	2580428	KIDNTUT13	157262F1 (THP1PLB02), 1914234X29C1 (PROSTUT04), 1914467X12C1 (PROSTUT04), 1914467X13C1 (PROSTUT04), 1915166X14C1 (PROSTUT04), 2580428H1 (KIDNTUT13), SBKA01222F1
29	81	3397189	UTRSNOT16	759108R6 (BRAITUT02), 1911587T6 (CONNTUT01), 3397189H1 (UTRSNOT16)
30	82	4881249	UTRMTMT01	080470R1 (SYNORAB01), 998242R6 (KIDNTUT01), 4549519H1 (HELAUNT01), 4881249H1 (UTRMTMT01), SXAE01512V1, SXAE02289V1, SXAE00433V1
31	83	431871	EOSINOT03	431871H1 (BRAVUNT02), 460185R1 (KERANOT01), 636514F1 (NEUTGMT01), 1975990T6 (PANCTUT02), 2212046H1 (SINTFET03), 2257310R6 (OVARTUT01), 2300180R6 (BRSTNOT05), 4884920F6 (LUNLTMT01), SCEA00887V1
32	84	526155	EOSINOT02	526155H1 (EOSINOT02), 794168R6 (OVARNOT03), 1260927R1 (SYNORAT05), 1975556F6 (PANCTUT02), 5157385H1 (BRSTTMT02)
33	85	676234	CRBLNOT01	676234H1 (CRBLNOT01), 2241232F6 (PANCTUT02), 2241232T6 (PANCTUT02), 2824092H1 (ADRETUT06), 4248435T6 (BRABDIT01)
34	86	720145	SYNOOAT01	433978H1 (THYRNOT01), 720145H1 (SYNOOAT01), 720145R6 (SYNOOAT01), 2107540T6 (BRAITUT03), 4722278H1 (COLCTUT02)
35	87	1001951	BRSTNOT03	1001951H1 (BRSTNOT03), 1001951R6 (BRSTNOT03), SXYA00708V1, SXYA01879V1, SXYA00520V1, SXYA00731V1, SXYA00926V1
36	88	1243349	LUNGNOT03	050083X316F1 (CHAONOT01), 050083X326F1 (CHAONOT01), 050083X346F1 (CHAONOT01), 050083X350F1 (CHAONOT01), 1243349H1 (LUNGNOT03), 2751089R6 (THP1AZS08), 3773254F6 (BRSTNOT25), 3997530H1 (PROSBPS05), g844357, g1940784, g4539083

Table 1 (cont.)

37	89	1338201	COLNNOT13	256461H1 (HNT2RAT01), 1338201H1 (COLNNOT13), 1338201X12 (COLNNOT13), 1338201X18 (COLNNOT13), 1338201X21 (COLNNOT13), 2078127H1 (ISLTNOT01), g777838, g1146680, g1406379
38	90	1405141	LATRTUT02	189682R6 (CARDNOT01), 551762R6 (SCORNOT01), 1405141X302D1 (LATRTUT02), 1459886X16C1 (COLNFET02), 2601416H1 (UTRSNOT10), 2836108H2 (TLYMNOT03), 3031895F6 (TLYMNOT05), 3127628H1 (LUNGTUT12), 3402733H1 (ESOGNOT03), 4289784F6 (BRABDIR01), 4339406H1 (BRAUNOT02), 4712515H1 (BRAIHCT01), 4746879H2 (SMCRUNT01), 5091792F6 (UTRSTMR01), 5679882H1 (BRAENOT02), 5927661H1 (BRAIFET02)
39	91	1686305	PROSNOT15	499154R6 (NEUTLPT01), 1686305F6 (PROSNOT15), 1686305H1 (PROSNOT15), 2306450R6 (NGANNOT01), 2446232F6 (THP1NOT03), 2446232T6 (THP1NOT03), 3050482H1 (LUNGNOT25), 3694303F6 (LUNGNOT35), 3825239H1 (BRAIHCT01), 3931022H1 (PROSTUT09), 4383527H1 (BRAVUTT02)
40	92	1688972	PROSTUT10	878019H1 (LUNGAST01), 1255436F2 (MENITUT03), 1330287F1 (PANCNOT07), 1400064F6 (BRAITUT08), 1688972H1 (PROSTUT10), 2018742F6 (THP1NOT01), 2047754X12F1 (SININOT01), 3002925H1 (TLYMNOT06), 3744192H1 (THYMNOT08)
41	93	1812494	PROSTUT12	1322590F6 (BLADNOT04), 1684555F6 (PROSNOT15), 2120930H1 (BRSTNOT07), 2266093H1 (UTRSNOT02), 2631470F6 (COLNTUT15), 3980110H1 (LUNGTUT08), 5115462H1 (ENDITXT01), SADA00912R1
42	94	2013853	TESTNOT03	2013853H1 (TESTNOT03), 2013853R6 (TESTNOT03), SXBC01227V1, SCSA04222V1
43	95	2284925	BRAINON01	464655X11 (LATRNOT01), 464655X12 (LATRNOT01), 464655X28 (LATRNOT01), 482019X21 (HNT2RAT01), 1443611R1 (THYRNOT03), 1443611X22 (THYRNOT03), 2284925H1 (BRAINON01), 2882173F6 (UTRSTUT05), 3485205F6 (KIDNNOT31), 3485205T6 (KIDNNOT31), SAAB00144R1

Table 1 (cont.)

44	96	2376728	ISLTNOT01	413593R6 (BRSTNOT01), 823803R1 (PROSNOT06), 860037R1 (BRAITUT03), 1282102F1 (COLNNOT16), 1733518F6 (BRSTTUT08), 2376728F6 (ISLTNOT01), 2376728H1 (ISLTNOT01), 2937285F6 (THYMFET02), 3108296H1 (BRSTTUT15), 3212546H1 (BLADNOT08), 3462704H1 (293TF2T01)
45	97	2790762	COLNTUT16	126628F1 (LUNGNOT01), 126628R1 (LUNGNOT01), 2790762F6 (COLNTUT16), 2790762H1 (COLNTUT16), 4002872H1 (HNT2AZS07), g678705
46	98	2869164	THYRNOT10	1607765F6 (LUNGNOT15), 2869164F6 (THYRNOT10), 2869164H1 (THYRNOT10), 2869164T6 (THYRNOT10), 2890205H1 (LUNGFET04), 2891521F6 (LUNGFET04), 3094580X305D1 (CERVNOT03)
47	99	3317629	PROSBPT03	3166243H1 (SATABT007), 3317629F6 (PROSBPT03), 3421114X302F1 (UCMCNOT04), 4635773F6 (MYEPTXT01), 4635773T6 (MYEPTXT01)
48	100	3870488	BMARNOT03	1670688F6 (BMARNOT03), 3039406T6 (BRSTNOT16), 3870488H1 (BMARNOT03), 4773630H1 (BRAQNOT01)
49	101	3886318	UTRSNOT05	198182F1 (KIDNNOT02), 474711R1 (MMLR1DT01), 733227R1 (LUNGNOT03), 1236870F1 (LUNGFET03), 1502818F1 (BRAITUT07), 3742588H1 (THYMNOT08)
50	102	4043934	LUNGNOT35	4043934F6 (LUNGNOT35), 4043934H1 (LUNGNOT35), g1664159, g2114678, g3665589
51	103	4371445	THYMNOT11	4371445F6 (THYMNOT11), 4371445H1 (THYMNOT11), 4371445T6 (THYMNOT11), g691417
52	104	5527925	KIDNNOT34	878842R1 (THYRNOT02), 1662614F6 (BRSTNOT09), 1820183F6 (GBLATUT01), 2275208H1 (PROSNON01), 2864564H1 (KIDNNOT20), 2890511H1 (LUNGFET04), 4312193H1 (BRAFNOT01), 5175111F6 (EPIBTXT01), 5876074H1 (BRAUNOT01)

Table 2

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences and Motifs	Homologous Sequences	Analytical Methods and Databases
1	446	T24 T144 S251 S384 S404 T114 T118 T121 T172 S181 S247 Y53 Y422	N117 N232	SH3 domain: E387-I441	g2232009, thyroid hormone responsive protein [Rattus norvegicus]. Shah, G.N. et al. (1997) Biochem. J. 327:617-23.	BLAST - GenBank BLAST - DOMO BLIMPS - BLOCKS BLIMPS - PRINTS HMMER - PFAM MOTIFS
2	340	T26 S51 T146 S211 S270 S308 S73 S277 S317 Y71		SH2 domain: W240-Y316	g3738265 SH2 domain-containing protein [Mus musculus]	BLAST - GenBank BLAST - DOMO BLIMPS - PRINTS HMMER - PFAM MOTIFS
3	353	T45 S232 T353 T78 S88 S163 S176 T222 S240 S284 S302 T326 S338 S116 S120 T154 S226 S295 S337		Pleckstrin homology domains: T247-T353 G4-H104 S120-K250	g5381422 pleckstrin 2 [Homo sapiens]	BLAST - GenBank BLAST - PRODOM HMMER - PFAM MOTIFS
4	593	S230 S415 T84 T115 S214 S231 S309 S355 S372 T377 T387 S529 S580 S5 T36 S41 S90 S205 T263 S264 T343 T371 S410 S445 S483 S528 T547	N19 N542	SH3 domain: L453-L507 EPS8 region - SH3/phosphorylation domain: S2-P395	g309217 Eps8 (EGF receptor kinase substrate) [Mus musculus]	BLAST - GenBank BLAST - PRODOM HMMER - PFAM MOTIFS

Table 2

5	358	T42 S82 T204 T233 S261 T271 T279 S285 S330 S55 T102 S153 S254 S353	N338	Ankyrin repeat: G40-G67	g485107 similar to ankyrin repeat region [C. elegans]	BLAST - GenBank HMMER - PFAM MOTIFS
6	749	S137 T401 S406 T407 S580 T29 S140 S148 S149 S287 T336 S342 S360 S511 S551 T627 T29 S104 T368 S480 T616 Y141 Y303	N147 N392 N453 N640	Transmembrane domain: W280-I297 SH3 domain: R483-L537 Probable rabGAP domains: I159-P168 Y200-G205	g1519685 contains similarity to SH3 domains [C. elegans].	BLAST - GenBank BLIMPS - PRINTS BLIMPS - PFAM HMMER - PFAM HMMER MOTIFS
7	139	T51 T113 S106	N31		g169306 calmodulin [Phytophthora infestans]	BLAST - GenBank
8	539	S52 S84 T114 S186 S430 T468 S15 S110 S241 S307 S309 S353 S362 S363 S389 S485 S118 S169 S181 S210 T319 S385 T434 T523 Y208 Y305	N533	Pleckstrin homology domain: R192-A291	g4151807 membrane-associated guanylate kinase-interacting protein 2 (Maguin-2) [Rattus norvegicus]	BLAST - GenBank HMMER - PFAM MOTIFS
9	319	S169 S214 S233 S240 S150	N126	Tumor necrosis factor and nerve growth factor receptors - Conserved domain containing six cysteines: L166-C204	g2809400 Sprouty 2 (antagonist of FGF signaling) [Homo sapiens]	BLAST - GenBank HMMER - PFAM MOTIFS

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences and Motifs	Homologous Sequences	Analytical Methods and Databases
10	747	T194 T344 T561 S655 S45 T58 T60 T74 T81 T171 S287 T294 S446 T526 S608 T610 T733 S126 S133 T165 S170 T190 S234 T251 T429 S470 S492 T522 S546 S735 S741 Y504 Y543	N32 N54 N533 N642		g550420 trg (transcript negatively regulated by thyroid stimulating hormone) [Rattus norvegicus]	
11	266	S62 T76 T183 S222 S4 T5 S256 S260 Y179	N47	Diacylglycerol/phorbol ester binding domain: E177-N223		PROFILESCAN HMMER - PFAM MOTIFS
12	345	T87 S131 S213 T241 S299 S323 T34 T69 T223 S307	N40 N70	Annexin domain: G58-L110 L122-R143 I137-L182 L262-F316 E311-D326 A327-C340	**g3688370, annexin 31 (annexin XXXI) [Homo sapiens]. Morgan, R.O. and Fernandez, M.P. (1998) FEBS Lett. 434:300-304.**	BLAST - GenBank HMMER - PFAM BLIMPS - BLOCKS BLIMPS - PRINTS MOTIFS
13	437	S40 T66 T79 S93 T241 T289 S305 S342 T375 S47 S270 S362 T371 T393			g685183 NGD5 gene product (regulated by opioid treatment) [Murinae gen. sp.]	BLAST - GenBank MOTIFS

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences and Motifs	Homologous Sequences	Analytical Methods and Databases
14	441	S333 S419 T10 T24 T322 S403 S407 S422 T453 S33 S270 S329 T352 S487		Ankyrin repeats: G46-N73 G80-D107	g6460678 ankyrin-related protein [Deinococcus radiodurans].	BLAST - GenBank HMMER - PFAM MOTIFS
15	487	S31 T51 S62 T220 T237 T254 T427 S453 T471 S482 T483 T95 S182	N242 N481	Signal peptide: M1-A28 Histidine acid phosphatase domains: R88-T95 K311-W323 Acid phosphatase-like region: E75-S484	g4105496 multiple inositol polyphosphate phosphatase [Mus musculus].	BLAST - GenBank BLAST - PRODOM BLIMPS - BLOCKS HMMER SPSCAN MOTIFS
16	282	S25 T125 T157 T203 S31 S46 S107 S133 S194 S218 S257	N17 N74 N216		g688297 VDUP1 (1,25-dihydroxy-vitamin D-3 up-regulated polypeptide [Homo sapiens].	BLAST - GenBank MOTIFS
17	581	T147 T327 S477 S41 T119 T123 T129 T209 S232 S243 S257 S299 S341 S347 T366 S371 S142 S220 S223 S237 S276 S323 S399 T472 T487 S518	N221 N358		**g6013191, activating signal cointegrator 1 [H. sapiens]. Kim, H.J.et al. (1999) Mol. Cell. Biol. 19:6323-6332.**	BLAST - GenBank MOTIFS

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences and Motifs	Homologous Sequences	Analytical Methods and Databases
18	530	S23 T46 S219 S221 T267 T268 S290 S303 T370 T382 S406 S446 T2 S31 S195 S339 S358 T375 S379 S399 T424 T445 T504	N43 N99	Signal peptide: M1-S23 WW/rsp5/WWP repeat domain: E123-P153 Trehalase domains: P80-T90 E129-N142	g1255031 FBP 30 (formin binding protein 30) [Mus musculus]	BLAST - GenBank SPSCAN HMMER - PFAM BLIMPS - BLOCKS MOTIFS
19 (034159)	475	S264 T5 T9 S33 S163 S171 S211 S217 S241 T267 S343 S370 T386 S472 S16 S110 S111 S151 S152 S246 T260 S264 T405	N15 N62 N101 N291 N384 N443	Pleckstrin M79-D189 GTPase activator K248-A459	g35013 n-chimaerin	Motifs BLAST_GENBANK HMMER_PFAM BLIMPS_PRINTS BLIMPS_PFAM BLAST_PRODOM BLAST_DOMO
20 (129023)	368	S8 S54 S70 S99 T158 S159 S253 S361 S30 T152 S308	N24 N68 N359	Signal peptide: M1-Q25 WW (signal transduction associated) domain: Y61-P75		Motifs SPSCAN BLIMPS_PRINTS
21 (1358940)	476	S104 S182 T343 S122 T148 T157 T197 S205 T360 S429 T467 T133 T269 T292 T323 S339		EF-hand Calcium binding domain: D231-D421	g3297882 atopy-related autoantigen CALC [H. sapiens].	Motifs BLAST_GENBANK HMMER_PFAM BLAST_PRODOM

Table 2 (cont.)

22 (1682320)	171	T70 T151 S97 Y11 Y24		Leucine zipper: L38-L59 Peptidyl-Prolyl Cis-Trans Isomerase CYP6: L59-F170	g1354207 rof1 FK506 binding protein	Motifs BLAST_GENBANK BLAST_PRODOM BLAST_DOMO
23 (1728263)	163	S16 S39 S56 T101 T112 T131 S148 Y92	N70	EF-hand calcium binding domain: D140-F152	g21209 caltractin **[Scherffelia dubia]**	Motifs BLAST_GENBANK BLAST_PRODOM
24 (1867626)	354	T230 T148 T252 S306 S315 T328 S8 T20 T27 S40 S71 T189 T244 T259 T288	N58 N64 N146 N250	Leucine zipper: L326-L347 ATP-Binding motif: E93-E320 Vasodilator-Stimulated Actin-Binding Phosphoprotein motif: M1-A109	g3834607 homer-1b [Mus musculus]	Motifs BLAST_GENBANK BLAST_PRODOM
25 (1990126)	365	T36 S47 S191 T198 S200 T359 T56 T124 S307 Y80 Y155	N189 N264 N297 N320	Src homology domain 3: R308-L364	g1407657 endophilin II	Motifs BLAST_GENBANK HMMER_PFAM BLIMPS_PRINTS BLAST_DOMO
26 (2104180)	274	T71 S126 T137 S230 S251 T7 S141 S155 Y152	N56	Protein Kinase C2 domain: L55-H135	g3876326 similar to protein kinase C2	Motifs BLAST_GENBANK HMMER_PFAM
27 (2122241)	129	T11 S24 S58 T100 S112 T89		Nascent polypeptide-associated complex alpha chain: G39-T128		Motifs BLAST_DOMO
28 (2580428)	626	S84 S93 S192 S278 T411 S10 S18 T114 S302 S482	N293 N577 N599	Interferon-gamma inducible protein motif: M1-M115, C522-A574	**g4886493 and g6942315, [H. sapiens].**	Motifs BLAST_PRODOM

Table 2 (cont.)

29 (3397189)	157	S7	N97	Signal peptide: M1-S29 Glycosyl hydrolase: L62-L137 Beta D Galactosidase: R28-L153	g2547317 lysosomal beta-galactosidase **WO9914328**	Motifs BLAST_GENBANK SPSCAN HMMER BLIMPS_BLOCKS BLAST_PRODOM
30 (4881249)	383	T7 T26 S90 T62 T81 S102 T363 S3 T210 T256 T286 Y158	N70 N190 N223 N289	WWP (Signal transduction associated proline binding domain):L201-P230	g5059333 ubiquitin ligase	Motifs BLAST_GENBANK HMMER_PFAM BLIMPS_PRINTS
31	478	S186 S202 S270 S354 S455 S9 S94 T175		Signal peptide: M1-A64 Ankyrin repeat: D36-E63 Ankyrin repeat protein domain: Q111-Y174; C285-V447	**g1204166, hypothetical Ank-repeat/BTB-domain protein [Schizosaccharomyces pombe].**	MOTIFS SPSCAN HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-PRODOM
32	275	S259 T74 T173 S186 T231 S21 T63 T219 S255 S267			COP9 complex subunit 7b [Mus musculus] g3309176	BLAST-Genbank MOTIFS
33	217	T4 T106 S209		Signal peptide: M1-C25 Transmembrane domains: A82-T100; R116-I34 Claudin signature: T21-W30; G49-V55 Q63-L73; D146-V152	claudin-9 protein [Mus musculus] g4325296	BLAST-Genbank MOTIFS SPSCAN HMMER BLIMPS-PRINTS

Table 2 (cont.)

34	74	S6 T58 S54		TPR domain: Y18-P46		MOTIFS HMMER-PFAM BLIMPS-PRODOM
35	367	S309 S24	N240	Transmembrane domain: L257-T277 Armadillo/beta-catenin repeat: 219-252; L252-L265		MOTIFS HMMER BLIMPS-PFAM
36	1113	T17 S43 S609 T755 T52 T215 S239 S287 T307 T313 S504 S510 S535 T536 S635 S688 S804 S812 T856 S863 T884 S938 T983 S996 S1004 S5 T196 S353 S433 T550 S592 S593 S727 T748 S762 S839 T928 S944 T952 T968 S1074 Y23 Y134	N175 N323 N365 N633 N724	PDZ domains: V53-E135; E152-D237 L252-H335; E472-D560 H573-D657; T673-Q754 K989-N1070 SH3 domain repeat: G98-K111 SH3 domain protein signature: V153-G249 GLGF domain: L676-K752	AMPA receptor interacting protein GRIP [Rattus norvegicus] g1890856	BLAST-Genbank MOTIFS HMMER-PFAM BLIMPS-PFAM BLIMPS-PRODOM BLAST-PRODOM BLAST-DOMO
37	511	S147 S88 S136 T228 T320 S467 T15 T81 T118 T168 S281 S289 S311 S354 S455 T461 T480 T494 Y16 Y114	N86 N116 N315 N316 N355 N403 N425 N429 N478	SH3 domain: Q342-L400	g6563258, insulin receptor tyrosine kinase substrate [Homo sapiens].	BLAST-Genbank MOTIFS HMMER-PFAM

Table 2 (cont.)

38	1177	S421 T936 T96 T121 S164 S209 T256 S277 S325 S374 S388 T397 S435 S443 T456 T519 S662 T669 S727 T901 S983 S1114 S14 T70 S307 S331 S416 S545 T565 S609 T626 T703 S804 S845 S853 S867 T921 S972 T1021 S1108 Y214 Y879 Y171	N84 N1112	Armadillo beta-catenin repeat: I196-L205	trg [Rattus norvegicus] g550420	BLAST-Genbank MOTIFS BLIMPS-PFAM
39	665	S245 T358 S480 T76 S110 S119 S121 T266 S284 S481 S521 S561 S632 S654 S655 S72 S73 S130 T171 S205 T411 S428 T475 S476 T491 S513 S523 T634 Y165 Y567 Y578	N197 N479	TPR domains: L136-P164; Y204-P232 E285-G313; P319-G347 F353-P381 TPR repeat: K137-E252; K286-K395	**g6272680, TPR-containing protein involved in spermatogenesis TPIS [Mus musculus]. Takaishi, M. and Huh, N.H. (1999) Biochem. Biophys. Res. Commun. 264:81-85.**	BLAST-Genbank MOTIFS HMMER-PFAM BLIMPS-PRODOM BLAST-DOMO
40	125	T119 T67		Signal peptide: M1-A53 SH3 domain: R68-L124 R68-A78; K112-L124		MOTIFS HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS

Table 2 (cont.)

Poly-peptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Motifs, and Domains	Homologous Sequences	Analytical Methods and Databases
41	366	S43 S45 T102 S157 T202 T220 S293 S219 T256 T325 S350 Y237		Signal peptide: M1-S30 Ankyrin repeat: G174-S206	**g289693, homology with isopentenyl-diphosphate-delta-isomerase; [C. elegans]. Sulston, J. et al. (1992) Nature 356:37-41.**	MOTIFS SPSCAN HMMER-PFAM BLIMPS-PFAM
42	173	S16 S42 S48 T67 S100 S111 S152 S86	N126	EF Hands: E22-R53; L57-F85 K94-M122; L135-L163 S-100/IcaBP type calcium binding protein signature: L6-E57; L132-K168 Recoverin family signature: V61-T82; S86-D105 Calmodulin repeat: R25-I79; L119-S157	calcineurin B-like protein (CBLP) [Rattus norvegicus] g220688	BLAST-GenBank MOTIFS HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS PROFILESCAN BLAST-DOMO
43	761	S227 S293 S393 S19 S43 T149 T161 S277 T346 T370 T415 T529 T572 S630 T683 S711 T746 S74 S196 S252 S283 S300 T444 T472 T591 S754 Y589	N117 N467 N492 N555	3'5'-cyclic nucleotide phosphodiesterase domain: Y490-H729 D418-W744 3'5'-cyclic nucleotide phosphodiesterase signature: L2-H56; L449-H485 Y490-H501; L516-D556 T572-E610; D657-S711	CAMP-specific cyclic nucleotide phosphodiesterase PDE8 [Mus musculus].	BLAST-GenBank MOTIFS HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS PROFILESCAN BLAST-PRODOM BLAST-DOMO

Table 2 (cont.)

Poly-peptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Motifs, and Domains	Homologous Sequences	Analytical Methods and Databases
44	249	S16 S89 T115 S212 S239 T12 T117 S137 S187 S197 S230 Y208	N84	Pleckstrin homology domain: V35-T131 Rho-GEF domain: L36-C178; E118-D245 FYVE zinc finger: N59-Y64; R171-C183 R202-S212	**g3292902, PUTATIVE RHO/RAC GUANINE NUCLEOTIDE EXCHANGE FACTOR [H. sapiens].**	BLAST-GenBank MOTIFS HMMER-PFAM BLIMPS-PFAM BLAST-PRODOM
45	247	S109 S44 S53 S123 T138 S167 S95 T98 S127 T220	N90		putative phosphatidyl-inositol 3-kinase [Carassius auratus] g4001815	BLAST-GenBank MOTIFS
46	316	S313 S201 T223 T262 Y186 Y270			g3811347, cytosolic phospholipase A2 beta [Homo sapiens].	BLAST-GenBank MOTIFS
47	334	T119 S97 T182 T244 S316 S317 S324 S60 T72 S97 T179 S187 S290 Y52 Y323	N58 N322	Fes/CIP4 homology domain: G8-L98 SH3 domain/division control protein signature: F6-F287	macrophage actin-associated-tyrosine-phosphorylated protein [Mus musculus] g3947712	BLAST-GenBank MOTIFS HMMER-PFAM BLAST-PRODOM
48	113	T65 S66 T43		SH3 domain: K34-L90	SLP-76 associated protein (TCR-stimulated PK substrate) [Homo sapiens] g2072873	BLAST-GenBank MOTIFS HMMER-PFAM BLIMPS-PRINTS

Table 2 (cont.)

Poly-peptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Motifs, and Domains	Homologous Sequences	Analytical Methods and Databases
49	264	S18 T76 T163 S181 S167 S223		Wilm's tumor protein signature: D97-P111	SH3 domain binding protein [Rattus norvegicus] g1185397 (P-value= 4.6x10-8).	BLAST-GenBank MOTIFS BLIMPS-PRINTS
50	185	T24 S81 S149 S151 S160 S162 S75 S99 S177 Y176		EF-hands: K101-L129; L143-S171 Recoverin family signature: I23-G42; S93-N112 Calcium binding protein signature: E12-Y104	g1848271, Calcium and integrin binding protein CIB [Homo sapiens]	BLAST-GenBank MOTIFS HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-PRODOM
51	72	T18 S25 T20		Synapse-associated SH3 domain protein signature: M13-E67	homolog of Drosophila discs large protein isoform 1 [Homo sapiens] g558438 (P-value= 7.9x10-9).	BLAST-GenBank MOTIFS BLAST-PRODOM
52	434	S123 T128 S418 S94 T105 S159 S205 T291 S308 S314 T326 T358 S383 S406 S84 T128 T212 Y220	N216 N231	Signal peptide: M1-A50 EF hand: I366-R394 Recoverin family signature: V370-L391	similar to EF hand [C. elegans] g3875264.	BLAST-GenBank MOTIFS SPSCAN HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS

Table 3

Nucleotide SEQ ID NO:	Selected Fragments	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
53	543-587	Reproductive (0.211) Developmental (0.158) Nervous (0.158)	Cancer (0.421) Cell Proliferation (0.263) Inflammation (0.211)	PBLUESCRIPT
54	273-317 651-695	Nervous (0.462) Gastrointestinal (0.385) Cardiovascular (0.077) Developmental (0.077)	Cancer (0.538) Cell Proliferation (0.308) Inflammation (0.154)	PSPORT1
55	110-154	Developmental (0.174) Gastrointestinal (0.174) Reproductive (0.174)	Cell Proliferation (0.435) Cancer (0.261) Inflammation (0.174)	pINCY
56	273-317 1461-1505	Gastrointestinal (0.821) Reproductive (0.143) Developmental (0.036)	Cancer (0.607) Inflammation (0.286) Cell Proliferation (0.036)	pINCY
57	595-639	Reproductive (0.313) Nervous (0.217) Hematopoietic/Immune (0.120)	Cancer (0.482) Inflammation (0.217) Cell Proliferation (0.169)	pINCY
58	703-747 1297-1341	Reproductive (0.250) Nervous (0.205) Gastrointestinal (0.125)	Cancer (0.509) Cell Proliferation (0.196) Inflammation (0.196)	pINCY
59	417-461	Nervous (0.300) Cardiovascular (0.200) Reproductive (0.200)	Inflammation (0.300) Trauma (0.300) Cancer (0.200) Cell Proliferation (0.200)	pINCY
60	1189-1233	Nervous (1.000)	Neurological (0.500) Trauma (0.333)	PSPORT1
61	272-316	Reproductive (0.314) Gastrointestinal (0.186) Nervous (0.157)	Cancer (0.529) Inflammation (0.200) Cell Proliferation (0.129)	PSPORT1
62	273-317 2055-2099	Hematopoietic/Immune (0.333) Reproductive (0.238) Gastrointestinal (0.167)	Inflammation (0.452) Cancer (0.333) Trauma (0.143)	PSPORT1
63	1-34	Reproductive (0.256) Nervous (0.188) Gastrointestinal (0.120)	Cancer (0.504) Inflammation (0.203) Cell Proliferation (0.195)	PSPORT1

Table 3 (cont.)

Nucleotide SEQ ID NO:	Selected Fragments	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
64	489-533	Reproductive (0.312) Gastrointestinal (0.125) Nervous (0.125)	Cancer (0.438) Cell Proliferation (0.375) Inflammation (0.188)	pINCY
65	273-317	Reproductive (0.265) Nervous (0.224) Developmental (0.102)	Cancer (0.469) Cell Proliferation (0.286) Inflammation (0.204)	pINCY
66	1028-1072	Cardiovascular (0.286) Nervous (0.200) Reproductive (0.200)	Cancer (0.429) Cell Proliferation (0.171) Inflammation (0.171)	pINCY
67	325-369	Reproductive (0.222) Nervous (0.194) Cardiovascular (0.167) Gastrointestinal (0.167)	Cancer (0.472) Cell Proliferation (0.333) Inflammation (0.139)	pINCY
68	921-965	Endocrine (0.250) Musculoskeletal (0.250) Reproductive (0.250) Urologic (0.250)	Cancer (0.750) Trauma (0.250)	pINCY
69	1029-1073	Reproductive (0.216) Gastrointestinal (0.176) Hematopoietic/Immune (0.157)	Cancer (0.510) Inflammation (0.275) Cell Proliferation (0.118)	pINCY
70	1405-1449	Hematopoietic/Immune (0.200) Nervous (0.200) Gastrointestinal (0.160) Reproductive (0.160)	Cancer (0.360) Inflammation (0.360) Cell Proliferation (0.200)	pINCY
71	280-324	Hematopoietic/Immune (0.500) Gastrointestinal (0.092) Reproductive (0.092)	Cancer (0.364) Inflammation (0.295) Cell proliferation (0.205)	pBLUESCRIPT
72	380-424	Reproductive (0.227) Gastrointestinal (0.205) Cardiovascular (0.114)	Cancer (0.455) Inflammation (0.364) Trauma (0.045)	pBLUESCRIPT

Table 3 (cont.)

73	433-477	Nervous (0.241) Reproductive (0.231) Gastrointestinal (0.130)	Cancer (0.398) Inflammation (0.333)	pINCY
74	786-830	Reproductive (0.342) Nervous (0.210)	Cancer (0.474) Cell proliferation (0.184) Inflammation (0.105)	pINCY
75	1-47	Gastrointestinal (0.286) Reproductive (0.286) Developmental (0.143) Hematopoietic/Immune (0.143)	Cancer (0.571) Cell proliferation (0.286) Inflammation (0.143)	pINCY
76	380-424	Nervous (0.300) Reproductive (0.200)	Inflammation (0.400) Cancer (0.200) Cell proliferation (0.200)	pINCY
77	30-74	Gastrointestinal (0.222) Reproductive (0.222) Cardiovascular (0.153) Nervous (0.153)	Inflammation (0.375) Cancer (0.361) Cell proliferation (0.139)	pINCY
78	487-531	Nervous (0.300) Reproductive (0.183) Cardiovascular (0.117)	Cancer (0.433) Inflammation (0.200) Neurological (0.133)	pSPORT1
79	595-639	Reproductive (0.305) Nervous (0.179) Gastrointestinal (0.126)	Cancer (0.526) Inflammation (0.326) Cell proliferation (0.179)	pINCY
80	109-153	Reproductive (0.235) Hematopoietic/Immune (0.216) Nervous (0.157)	Cancer (0.529) Inflammation (0.255)	pINCY
81	109-153	Gastrointestinal (0.286) Musculoskeletal (0.286) Reproductive (0.286)	Cancer (0.571) Inflammation (0.286)	pINCY
82	163-207	Reproductive (0.424) Gastrointestinal (0.152) Nervous (0.121)	Cancer (0.424) Inflammation (0.242) Cell proliferation (0.182)	pINCY
83	496-540	Reproductive (0.242) Nervous (0.182) Hematopoietic/Immune (0.167)	Cancer (0.455) Inflammation/Trauma (0.364) Cell Proliferation (0.152)	PSPORT1
84	1022-1066	Reproductive (0.248) Nervous (0.208) Cardiovascular (0.136)	Cancer (0.464) Inflammation/Trauma (0.304) Cell Proliferation (0.184)	PSPORT1

Table 3 (cont.)

85	39-83	Nervous (0.286) Endocrine (0.143) Gastrointestinal (0.143) Hematopoietic/Immune (0.143) Reproductive (0.143)	Cancer (0.571) Inflammation/Trauma (0.286) Neurological (0.143)	PSPORT1
86	471-515	Hematopoietic/Immune (0.167) Musculoskeletal (0.167) Reproductive (0.167)	Cancer (0.556) Cell Proliferation (0.167) Inflammation/Trauma (0.167)	PSPORT1
87	595-639 982-1026	Reproductive (0.294) Cardiovascular (0.176) Gastrointestinal (0.176)	Cancer (0.706) Inflammation/Trauma (0.294) Cell Proliferation (0.118)	PSPORT1
88	1101-1163	Reproductive (0.625) Gastrointestinal (0.250) Cardiovascular (0.125)	Cancer (0.750) Inflammation/Trauma (0.250)	PSPORT1
89	1245-1289	Gastrointestinal (0.387) Reproductive (0.355) Cardiovascular (0.065)	Cancer (0.548) Inflammation/Trauma (0.323) Cell Proliferation (0.161)	pINCY
90	3720-3764	Nervous (0.328) Gastrointestinal (0.121) Reproductive (0.121)	Cancer (0.397) Inflammation/Trauma (0.310) Cell Proliferation (0.155)	pINCY
91	659-703 1622-1666	Hematopoietic/Immune (0.273) Nervous (0.182) Cardiovascular (0.121) Reproductive (0.121)	Cancer (0.455) Cell Proliferation (0.333) Inflammation/Trauma (0.303)	pINCY
92	104-148	Reproductive (0.310) Nervous (0.241) Developmental (0.138) Gastrointestinal (0.138)	Cancer (0.483) Inflammation/Trauma (0.241) Cell Proliferation (0.172)	pINCY
93	820-864	Reproductive (0.340) Cardiovascular (0.120) Nervous (0.120)	Inflammation/Trauma (0.440) Cancer (0.400) Cell Proliferation (0.160)	pINCY
94	504-554	Reproductive (1.000)	Inflammation/Trauma (1.000)	PBLUESCRIPT
95	198-242	Reproductive (0.424) Nervous (0.273)	Cancer (0.576) Inflammation/Trauma (0.182)	PSPORT1
96	307-351 712-756	Reproductive (0.412) Hematopoietic/Immune (0.137) Cardiovascular (0.118) Gastrointestinal (0.118)	Cancer (0.608) Inflammation/Trauma (0.275) Cell Proliferation (0.098)	pINCY

Table 3 (cont.)

97	433-477	Developmental (0.200) Reproductive (0.200) Cardiovascular (0.133) Gastrointestinal (0.133) Nervous (0.133)	Cell Proliferation (0.400) Cancer (0.333) Inflammation/Trauma (0.200)	pINCY
98	474-1018	Cardiovascular (0.190) Reproductive (0.190) Hematopoietic/Immune (0.143) Musculoskeletal (0.143)	Cancer (0.381) Inflammation/Trauma (0.333)	pINCY
99	422-466 998-1042	Hematopoietic/Immune (0.667) Reproductive (0.222) Developmental (0.111)	Inflammation/Trauma (0.556) Cancer (0.222) Cell Proliferation (0.222)	pINCY
100	444-488	Hematopoietic/Immune (0.455) Nervous (0.182) Cardiovascular (0.091)	Inflammation/Trauma (0.546) Cancer (0.182) Cell Proliferation (0.182)	pINCY
101	1578-1622	Reproductive (0.250) Nervous (0.170) Gastrointestinal (0.156)	Cancer (0.482) Inflammation/Trauma (0.345) Cell Proliferation (0.167)	pINCY
102	15-59	Cardiovascular (1.000)	Cancer (1.000)	pINCY
103	487-531	Hematopoietic/Immune (1.000)		pINCY
104	967-1011	Reproductive (0.235) Nervous (0.191) Gastrointestinal (0.147)	Cancer (0.515) Inflammation/Trauma (0.294) Cell Proliferation (0.118)	pINCY

Table 4

Nucleotide SEQ ID NO:	Library	Library Description
53	TESTNOT01	The library was constructed using RNA isolated from the testicular tissue of a 37-year-old Caucasian male, who died from liver disease. Patient history included cirrhosis, jaundice, and liver failure.
54	COLNNOT05	The library was constructed using RNA isolated from the sigmoid colon tissue of a 40-year-old Caucasian male during a partial colectomy. Pathology indicated Crohn's disease involving the proximal colon and including the cecum. The ascending and transverse colon displayed linear ulcerations and skip lesions. Transmural inflammation was present.
55	KIDNNOT09	The library was constructed using RNA isolated from the kidney tissue of a Caucasian male fetus who died at 23 weeks' gestation.
56	COLNFET02	The library was constructed using RNA isolated from the colon tissue of a Caucasian female fetus who died at 20 weeks' gestation.
57	CONNNOT01	The library was constructed using RNA isolated from mesentery fat tissue obtained from a 71-year-old Caucasian male during a partial colectomy and permanent colostomy. Family history included atherosclerotic coronary artery disease, myocardial infarction, and extrinsic asthma.
58	BRAINOT09	The library was constructed using RNA isolated from brain tissue removed from a Caucasian male fetus who died at 23 weeks' gestation.
59	ENDCNOT02	The library was constructed using RNA isolated from dermal microvascular endothelial cells removed from a 30-year-old Caucasian female.
60	HIPONON02	This normalized library was constructed using 1.13 million independent clones from a hippocampus library. RNA was isolated from the hippocampus tissue of a 72-year-old Caucasian female who died from an intracranial bleed. Patient history included nose cancer, hypertension, and arthritis. The normalization and hybridization conditions were adapted from Soares et al. (PNAS (1994) 91:9228).
61	OVARNOT02	The library was constructed using RNA isolated from ovarian tissue removed from a 59-year-old Caucasian female who died of a myocardial infarction. Patient history included cardiomyopathy, coronary artery disease, myocardial infarction, hypercholesterolemia, hypotension, and arthritis.
62	OVARNOT02	The library was constructed using RNA isolated from ovarian tissue removed from a 59-year-old Caucasian female who died of a myocardial infarction. Patient history included cardiomyopathy, coronary artery disease, myocardial infarction, hypercholesterolemia, hypotension, and arthritis.

Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Description
63	ADRETUT01	The library was constructed using RNA isolated from right adrenal tumor tissue removed from a 50-year-old Turkish male during a unilateral adrenalectomy. Pathology indicated a metastatic renal cell carcinoma that formed a circumscribed, spongy, hemorrhagic nodule situated in the region of the medulla. The patient presented with corticoadrenal insufficiency, incisional hernia, and non-alcoholic steato hepatitis. Patient history included renal cell carcinoma. Family history included liver cancer.
64	GBLANOT01	The library was constructed using RNA isolated from diseased gallbladder tissue removed from a 53-year-old Caucasian female during a cholecystectomy. Pathology indicated mild chronic cholecystitis and cholelithiasis with approximately 150 mixed gallstones. Family history included benign hypertension.
65	LUNGTUT09	The library was constructed using RNA isolated from lung tumor tissue removed from a 68-year-old Caucasian male during segmental lung resection. Pathology indicated invasive grade 3 squamous cell carcinoma and a metastatic tumor. Patient history included type II diabetes, thyroid disorder, depressive disorder, hyperlipidemia, esophageal ulcer, and tobacco use.
66	PONSAZT01	The library was constructed using RNA isolated from diseased pons tissue removed from the brain of a 74-year-old Caucasian male who died from Alzheimer's disease.
67	293TF1T01	The library was constructed using RNA isolated from a transformed embryonal cell line (293-EBNA) derived from kidney epithelial tissue. The cells were transformed with adenovirus 5 DNA.
68	ADRENOT14	The library was constructed using RNA isolated from adrenal gland tissue removed from an 8-year-old Black male who died from anoxia.
69	BRAVTXT03	The library was constructed using RNA isolated from treated astrocytes removed from the brain of a female fetus who died at 22 weeks' gestation. The cells were treated with tumor necrosis factor (TNF) alpha and interleukin 1 (IL-1), 10ng/ml each for 24 hours.

Table 4 (cont.)

70	293TF2T01	The library was constructed using RNA isolated from a treated, transformed embryonal cell line (293-EBNA) derived from kidney epithelial tissue. The cells were treated with 5-aza-2'-deoxycytidine and transformed with adenovirus 5 DNA.
71	THP1NOB01	Library was constructed using RNA isolated from cultured, unstimulated THP-1 cells. THP-1 (ATCC TIB 202) is a human promonocyte line derived from the peripheral blood of a 1-year-old Caucasian male with acute monocytic leukemia. RNA was isolated from 2x108 cells using GuSCN lysis, followed by DNAse treatment.
72	TESTNOT01	Library was constructed using RNA isolated from the testicular tissue of a 37-year-old Caucasian male, who died from liver disease. Patient history included cirrhosis, jaundice, and liver failure.
73	LUNGNOT09	Library was constructed using RNA isolated from the lung tissue of a 23-week-old Caucasian male fetus. The pregnancy was terminated following a diagnosis by ultrasound of infantile polycystic kidney disease.
74	PROSNOT15	Library was constructed using RNA isolated from diseased prostate tissue removed from a 66-year-old Caucasian male during radical prostatectomy and regional lymph node excision. Pathology indicated adenofibromatous hyperplasia. Pathology for the associated tumor tissue indicated an adenocarcinoma (Gleason grade 2+3). The patient presented with elevated prostate specific antigen (PSA). Family history included prostate cancer, secondary bone cancer, and benign hypertension.
75	PROSNOT14	Library was constructed using RNA isolated from diseased prostate tissue removed from a 60-year-old Caucasian male during radical prostatectomy and regional lymph node excision. Pathology indicated adenofibromatous hyperplasia. Pathology for the associated tumor tissue indicated an adenocarcinoma (Gleason grade 3+4). Family history included benign hypertension, cerebrovascular disease, and arteriosclerotic coronary artery disease.
76	SKINBIT01	Library was constructed using RNA isolated from diseased skin tissue of the left lower leg. Patient history included erythema nodosum of the left lower leg.
77	CORPNOT02	Library was constructed using RNA isolated from diseased corpus callosum tissue removed from the brain of a 74-year-old Caucasian male who died from Alzheimer's disease.
78	BRAITUT02	Library was constructed using RNA isolated from brain tumor tissue removed from the frontal lobe of a 58-year-old Caucasian male during excision of a cerebral meningeal lesion. Pathology indicated a grade 2 metastatic hypernephroma. Patient history included a grade 2 renal cell carcinoma, insomnia, and chronic airway obstruction. Family history included a malignant neoplasm of the kidney.

Table 4 (cont.)

79	BRSTNOT07	Library was constructed using RNA isolated from diseased breast tissue removed from a 43-year-old Caucasian female during a unilateral extended simple mastectomy. Pathology indicated mildly proliferative fibrocystic changes with epithelial hyperplasia, papillomatosis, and duct ectasia. Pathology for the associated tumor tissue indicated invasive grade 4, nuclear grade 3 mammary adenocarcinoma with extensive comedo necrosis. Family history included epilepsy, cardiovascular disease, and type II diabetes.
80	KIDNTUT13	Library was constructed using RNA isolated from kidney tumor tissue removed from a 51-year-old Caucasian female during a nephroureterectomy. Pathology indicated a grade 3 renal cell carcinoma. Family history included calculus of the kidney, colon cancer, and type II diabetes.
81	UTRSNOT16	Library was constructed using RNA isolated from uterine endometrial tissue removed from a 48-year-old Caucasian female during a vaginal hysterectomy, rectocele repair, and bilateral salpingo-oophorectomy. Pathology indicated chronic cervicitis, and the endometrium was weakly proliferative. Pathology for the associated tumor tissue indicated a single submucosal leiomyoma. Patient history included hyperlipidemia and meningitis. Family history included benign hypertension, hyperlipidemia, atrial fibrillation, atherosclerotic coronary artery disease, and type II diabetes.
82	UTRMTMT01	Library was constructed using RNA isolated from myometrial tissue removed from a 45-year-old Caucasian female during vaginal hysterectomy and bilateral salpingo-oophorectomy. Pathology indicated the myometrium was negative for tumor. Pathology for the associated tumor tissue indicated multiple (23) subserosal, intramural, and submucosal leiomyomata. Patient history included extrinsic asthma without status asthmaticus and normal delivery. Family history included cerebrovascular disease, depression, and atherosclerotic coronary artery disease.
83	EOSINOT03	This library was constructed using RNA isolated from pooled diseased eosinophils obtained from allergic asthmatic individuals.
84	EOSINOT02	This library was constructed using RNA isolated from pooled eosinophils obtained from allergic asthmatic individuals.
85	CRBLNOT01	This library was constructed using RNA isolated from the cerebellum tissue of a 69-year-old Caucasian male who died from chronic obstructive pulmonary disease. Patient history included myocardial infarction, hypertension, and osteoarthritis.
86	SYNOOAT01	This library was constructed using RNA isolated from the knee synovial membrane tissue of an 82-year-old female with osteoarthritis.
87	BRSTNOT03	This library was constructed using RNA isolated from diseased breast tissue removed from a 54-year-old Caucasian female during a bilateral radical mastectomy. Pathology for the associated tumor tissue indicated residual invasive grade 3 mammary ductal adenocarcinoma. Patient history included kidney infection and condyloma acuminatum.

		Family history included benign hypertension, hyperlipidemia, and a malignant neoplasm of the colon.
88	LUNGNOT03	This library was constructed using RNA isolated from lung tissue of a 79-year-old Caucasian male. Pathology for the associated tumor tissue indicated grade 4 carcinoma. Patient history included a benign prostate neoplasm and atherosclerosis.
89	COLNNOT13	This library was constructed using RNA isolated from ascending colon tissue of a 28-year-old Caucasian male with moderate chronic ulcerative colitis.
90	LATRTUT02	This library was constructed using RNA isolated from a myxoma removed from the left atrium of a 43-year-old Caucasian male during annuloplasty. Pathology indicated atrial myxoma. Patient history included pulmonary insufficiency, acute myocardial infarction, atherosclerotic coronary artery disease, hyperlipidemia, and tobacco use. Family history included benign hypertension, acute myocardial infarction, atherosclerotic coronary artery disease, and type II diabetes.
91	PROSNOT15	This library was constructed using RNA isolated from diseased prostate tissue removed from a 66-year-old Caucasian male during radical prostatectomy and regional lymph node excision. Pathology indicated adenofibromatous hyperplasia. Pathology for the associated tumor tissue indicated an adenocarcinoma (Gleason grade 2+3). The patient presented with elevated prostate specific antigen (PSA). Family history included prostate cancer, secondary bone cancer, and benign hypertension.
92	PROSTUT10	This library was constructed using RNA isolated from prostatic tumor tissue removed from a 66-year-old Caucasian male during radical prostatectomy and regional lymph node excision. Pathology indicated an adenocarcinoma (Gleason grade 2+3). Adenofibromatous hyperplasia was also present. The patient presented with elevated prostate specific antigen (PSA). Family history included prostate cancer and secondary bone cancer.
93	PROSTUT12	This library was constructed using RNA isolated from prostate tumor tissue removed from a 65-year-old Caucasian male during a radical prostatectomy. Pathology indicated an adenocarcinoma (Gleason grade 2+2). Adenofibromatous hyperplasia was also present. The patient presented with elevated prostate specific antigen (PSA).
94	TESTNOT03	This library was constructed using RNA isolated from testicular tissue removed from a 37-year-old Caucasian male, who died from liver disease. Patient history included cirrhosis, jaundice, and liver failure.
95	BRAINON01	This library was constructed and normalized from 4.88 million independent clones from a brain library. RNA was made from brain tissue removed from a 26-year-old Caucasian male during cranioplasty and excision of a cerebral meningeal lesion. Pathology for the associated tumor tissue indicated a grade 4 oligoastrocytoma in the right fronto-parietal part of the brain.

Table 4 (cont.)

96	ISLTNOT01	This library was constructed using RNA isolated from a pooled collection of pancreatic islet cells.
97	COLNTUT16	This library was constructed using RNA isolated from colon tumor tissue obtained from a 60-year-old Caucasian male during a left hemicolectomy. Pathology indicated an invasive grade 2 adenocarcinoma, forming a sessile mass. Patient history included thrombophlebitis, inflammatory polyarthropathy, prostatic inflammatory disease, and depressive disorder. Previous surgeries included resection of the rectum. Family history included atherosclerotic coronary artery disease and colon cancer.
98	THYRNOT10	This library was constructed using RNA isolated from the diseased left thyroid tissue removed from a 30-year-old Caucasian female during a unilateral thyroid lobectomy and parathyroid reimplantation. Pathology indicated lymphocytic thyroiditis.
99	PROSBPT03	This library was constructed using RNA isolated from diseased prostate tissue removed from a 59-year-old Caucasian male during a radical prostatectomy and regional lymph node excision. Pathology indicated benign prostatic hyperplasia (BPH). Pathology for the associated tumor indicated adenocarcinoma, Gleason grade 3+3. The patient presented with elevated prostate specific antigen (PSA), benign hypertension, and hyperlipidemia. Family history included cerebrovascular disease, benign hypertension and prostate cancer.
100	BMARNOT03	This library was constructed using RNA isolated from the left tibial bone marrow tissue of a 16-year-old Caucasian male during a partial left tibial ostectomy with free skin graft. Patient history included an abnormality of the red blood cells. Family history included osteoarthritis.
101	UTRSNOT05	This library was constructed using RNA isolated from the uterine tissue of a 45-year-old Caucasian female during a total abdominal hysterectomy and total colectomy. Pathology for the associated tumor tissue indicated multiple leiomyomas of the myometrium and a grade 2 colonic adenocarcinoma of the cecum. Patient history included multiple sclerosis and mitral valve disorder. Family history included type I diabetes, cerebrovascular disease, atherosclerotic coronary artery disease, malignant skin neoplasm, hypertension, and malignant neoplasm of the colon.

Table 4 (cont.)

102	LUNGNOT35	This library was constructed using RNA isolated from lung tissue removed from a 62-year-old Caucasian female. Pathology for the associated tumor tissue indicated a grade 1 spindle cell carcinoid forming a nodule. Patient history included depression, thrombophlebitis, and hyperlipidemia. Family history included cerebrovascular disease, atherosclerotic coronary artery disease, breast cancer, colon cancer,type II diabetes, and malignant skin melanoma.
103	THYMNOT11	This library was constructed using RNA isolated from thymus tissue removed from a 2-year-old Caucasian female during a thymectomy and patch closure of left atrioventricular fistula. The patient presented with congenital heart abnormalities. Patient history included double inlet left ventricle and a rudimentary right ventricle, pulmonary hypertension, cyanosis, subaortic stenosis, seizures, and a fracture of the skull base. Family history included reflux neuropathy.
104	KIDNNOT34	This library was constructed using RNA isolated from left kidney tissue obtained from an 8-year-old Caucasian male who died from an intracranial hemorrhage.

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	*ESTs:* Probability value= 1.0E-8 or less *Full Length sequences*: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises as least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	*ESTs:* fasta E value=1.06E-6 *Assembled ESTs*: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less *Full Length sequences:* fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and, if applicable, Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits for PFAM hits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Normalized quality score≥GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=3.5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. supra; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	